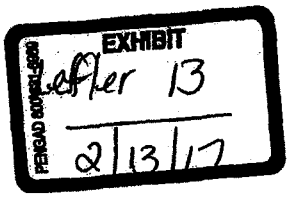
Exhibit F

ORIGINAL ARTICLE

The clinical and phenotypical assessment of seronegative villous atrophy; a prospective UK centre experience evaluating 200 adult cases over a 15-year period (2000–2015)



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ABSTRACT

Background Seronegative villous atrophy (SNVA) is commonly attributed to coeliac disease (CD). However, there are other causes of SNVA. More recently angiotensin-2-receptor-blockers (A2RBs) have been reported as an association but data on SNVA have been limited to centres evaluating complex case referrals and not SNVA in general.

Objectives To provide clinical outcomes and associations in a large prospective study overseeing all newcomers with SNVA.

Design Over a 15-year period (2000–2015) we evaluated 200 adult patients with SNVA at a UK centre. A diagnosis of either seronegative CD (SNCD) or seronegative non-CD (SN-non-CD) was reached. Baseline comparisons were made between the groups, with 343 seropositive CD subjects serving as controls.

Results Of the 200 SNVA cases, SNCD represented 31% (n=62) and SN-non-CD 69% (n=138). The human leucocyte antigen (HLA)-DQ2 and/or DQ8 genotype was present in 61%, with a 51% positive predictive value for SNCD. The breakdown of identifiable causes in the SN-non-CD group comprised infections (27%, n=54), inflammatory/immune-mediated disorders (17.5%, n=35) and drugs (6.5%, n=13; two cases related to A2RBs). However, no cause was found in 18% (n=36) and of these 72% (n=26/36) spontaneously normalised duodenal histology while consuming a gluten-enriched diet. Following multivariable logistic regression analysis an independent factor associated with SN-non-CD was non-white ethnicity (OR 10.8, 95% CI 2.2 to 52.8); in fact, 66% of non-whites had GI infections. On immunohistochemistry all groups stained positive for CD8-T-cytotoxic intraepithelial lymphocytes. However, additional CD4-T helper intraepithelial lymphocytes were occasionally seen in SN-non-CD mimicking the changes associated with refractory CD.

Conclusions Most patients with SNVA do not have CD, in particular those who are not white. Furthermore, a subgroup with no obvious aetiology will show spontaneous histological resolution while consuming gluten. These findings suggest caution in empirically prescribing a gluten-free diet without investigation.

Significance of this study

What is already known on this subject?
- Seronegative villous atrophy (SNVA) is a diagnostic and therapeutic dilemma.
- The causes of SNVA are vast but can be broadly grouped into seronegative coeliac disease (SNCD) and seronegative non-coeliac disease (SN-non-CD).
- To date no study has systematically evaluated all newcomers with SNVA.

What are the new findings?
- SNCD accounts for 31% of SNVA cases, with the remaining 69% due to SN-non-CD.
- A positive human leucocyte antigen DQ2 and/or DQ8 status is seen in 61% of SNVA cases; its positive predictive value for SNCD is roughly 51%.
- An independent risk factor associated with SN-non-CD is non-white ethnicity, suggestive of infective aetiology.
- Overall, almost one in five patients with SNVA will have no identifiable cause; reassuringly, the majority of these will spontaneously normalise duodenal histology despite undertaking a gluten challenge.

How might it impact on clinical practice in the foreseeable future?
- Individuals with SNVA should not be prescribed a gluten-free diet prior to further investigations. This is because of the wide differential diagnoses and that a subgroup with no obvious aetiology spontaneously normalises its duodenal histology while maintaining gluten intake.

INTRODUCTION

Coeliac disease (CD) affects 0.7–1% of the population and can be defined as a state of heightened immune response to ingested gluten in genetically susceptible individuals.[1 2] All patients with CD carry the human leucocyte antigen (HLA)-DQ2 and/or DQ8 genotypes, although these alleles are also present in approximately 40% of the general population.[3] A cast-iron diagnosis of CD can be made on the basis of demonstrating duodenal

villous atrophy in the presence of serum IgA endomysial and/or tissue transglutaminase antibodies.[4–6] This mode of presentation may be termed seropositive CD (SPCD) and following a systematic review accounts for approximately 93% of cases with villous atrophy,[7] although some international groups have reported a lower prevalence (table 1).[8–15]

With this in regard, diagnostic and therapeutic dilemmas occur when villous atrophy is found in the context of negative coeliac serology.[8–15] This clinical entity is termed seronegative villous atrophy (SNVA), the causes of which can be broadly grouped into CD or non-CD related.[16 17] The reasons for seronegative CD (SNCD) include patients who have reduced gluten intake prior to investigations,[18] lesser degrees of villous atrophy,[13] selective IgA deficiency,[19] immunosuppressive therapy or those with long-standing advanced CD within the spectrum of ulcerative jejunitis/enteropathy associated T cell lymphoma.[15] The causes of seronegative non-CD (SN-non-CD) are vast ranging from infective, inflammatory, immune-mediated and drug-related.[16 17] Such examples include autoimmune enteropathy,[20] bacterial overgrowth,[16] common variable immunodeficiency,[21] Crohn's disease,[22] gastroenteritis,[23] giardiasis,[24 25] graft versus host disease,[26] HIV enteropathy,[27] mycobacterium tuberculosis,[25 28] peptic duodentitis±*H. pylori*,[17 29–32] radiation enteritis,[33] tropical sprue[25 34] and Whipple's disease.[35] Medications include non-steroidal anti-inflammatory drugs,[36–38] azathioprine,[39] methotrexate,[40] mycophenolate mofetil[41] and, most recently, angiotensin-2-receptor-blockers (A2RBs), in particular olmesartan.[16 42–45] Finally, in some instances no unifying cause can be found and such patients are classified as idiopathic/unclassified sprue, the natural history of which is unknown.[16 17]

Studies attempting to evaluate diagnostic outcomes in SNVA have thus far been limited to a US centre overseeing complex case referrals from a wide catchment area.[16] In such circumstances a high prevalence of SNCD and olmesartan-related enteropathy has been reported, the latter accounting for a striking 22% of SNVA cases.[16] However, we hypothesise that this may not be reflective of SNVA as seen in routine GI practice. Furthermore, the clinical and histological phenotype of SNCD and SN-non-CD has not been established, nor how these entities contrast to the more conventionally seen SPCD. Such an evaluation may prove useful in understanding the spectrum of villous atrophy while also aiding clinicians towards the correct diagnosis when posed with the challenges of SNVA.

In light of this, the aim of our study was to provide a large comprehensive overview of all patients with SNVA seen at a UK centre over a 15-year period. Furthermore, we sought to identify differences between SNCD and SN-non-CD, using SPCD as controls.

MATERIAL AND METHODS

Setting

This study was carried out between the time periods of 2000 and 2015 at the Royal Hallamshire Hospital, Sheffield, South Yorkshire, UK. The hospital is located in northern England and provides a secondary/tertiary-care service to a population of 500 000 people. The unit undertakes approximately 6000 oesophagogastroduodenoscopies per year.

Participants

Over the 15-year period we prospectively recruited 200 consecutive adult patients presenting with SNVA. The identification of SNVA was based upon duodenal biopsies showing villous atrophy yet with negative serum IgA endomysial and tissue transglutaminase antibodies from the outset.

As for our control group we recruited 343 patients with SPCD diagnosed within the same department between the years 2005 and 2011.

Histology

Throughout the study period the gastroenterology department had a policy of taking four duodenal biopsy specimens from the second part of the duodenum in those with suspected malabsorption. All duodenal biopsy specimens were fixed in buffered formalin and embedded in paraffin wax. Standard 3 µm thick sections at three levels were stained with H&E. The duodenal biopsies were routinely reported by one of a team of seven GI histopathologists. Agreement was then performed by one of two expert GI histopathologists reviewing SNVA biopsy samples (coauthors SSC and PV). Intraepithelial lymphocytosis was defined as >25 per 100 enterocytes. Villous atrophy was identified according to the Marsh-Oberhuber criteria, using the most severe lesion present: Marsh 3a (partial villous atrophy, PVA); Marsh 3b (subtotal villous atrophy, SVA); or Marsh 3c (total villous atrophy, TVA).[46 47]

The groups were also assessed for differences in immunohistochemistry based on CD3 pan-lymphocyte marker and specific CD8-T cytotoxic and CD4-T helper intraepithelial lymphocyte expression.

Coeliac serology

The initial panel of coeliac serology testing was IgA based, with endomysial antibodies detected by immunofluoresence on primate oesophagus sections from The Binding Site (Birmingham, UK). IgA tissue transglutaminase antibodies were assayed by using ELISA kits (Aesku Diagnostics, Wendelsheim, Germany), with titres less than or equal to 15 U/mL taken as negative. Of note, our immunology department does not automatically test for immunoglobulin or total IgA levels when

Table 1 Studies where coeliac serology have shown low sensitivities in villous atrophy.

First author	Year	Country	No of villous atrophy cases	Positive coeliac serology, n (%)	Negative coeliac serology, n (%)
Rostami[8]	1999	Netherlands	69	42 (61%)	27 (39%)
Dickey[9]	2000	Northern Ireland	89	69 (78%)	20 (22%)
Dahele[10]	2001	Scotland	53	42 (79%)	11 (21%)
Dahele[11]	2001	Scotland	114	92 (81%)–99 (87%)	15 (11%)–22(19%)
Clemente[12]	2002	Italy	111	95 (86%)	16 (14%)
Abrams[13]	2004	USA	115	74 (64%)	41 (36%)
Collin[14]	2005	European multicentre	126	112 (89%)–118 (94%)	8 (6%)–14 (11%)
Salmi[15]	2006	Finland	177	151 (85%)	26 (15%)

Coeliac serology as defined by endomysial and/or tissue transglutaminase antibodies.

processing coeliac serology. Rather, these have to be specifically requested as does IgG coeliac serology.

Baseline characteristics

We collected baseline characteristic data on the SNVA and SPCD cohorts. Taking into consideration the potential aetiologies and clinical manifestations this included age, gender, ethnicity, city residence, clinical symptoms, past medical history, current medication, grading of villous atrophy, HLA-DQ2/8 status, as well as laboratory parameters in the form of haemoglobin, ferritin, folate, vitamin B_{12}, albumin, calcium, erythrocyte sedimentation rate and/or C reactive protein.

All the data (other than age) were inputted as categorical. This included converting numerical laboratory values into either within the normal or abnormal range, thereby overcoming the difficulties that arise over a 15-year period with departmental changes in testing kits and reference values.

Diagnostic workup for SNVA

All patients with SNVA were investigated in line with a systematic protocol, similar to that proposed by other expert groups, aiming to diagnose either SNCD or SN-non-CD (figure 1).[16 17] It is important to bear in mind that, despite several international guidelines on CD, there is no consensus on how to approach subjects with SNVA.[4–6] Some physicians may suggest a trial of a gluten-free diet (GFD) followed by clinical and histological reassessment.[4–6] However, this can be fraught with uncertainty given that up to 32% of patients with SN-non-CD report favourable clinical response to a GFD.[17] Furthermore, mucosal recovery in adult CD is slow with histological abnormalities often persisting beyond 2–5 years and in some cases never normalising.[48–50] Therefore, adopting such an approach in SNVA could potentially lead to unnecessary delays given the wide differential diagnoses. Hence, patients with SNVA in our study were asked to continue a gluten-containing diet until investigations were complete and a firm diagnosis reached. This approach is also useful in that it allows progression of villous atrophy and detectable serum antibodies in some cases of SNCD.[51]

Mortality

At the end of December 2015 mortality rates were calculated. Overall survival was calculated in years and defined as the time from diagnosis to death. Surviving patients were censored at the time of last follow-up.

Statistics

Statistical analysis was carried out using SPSS V.21.0 software (SPSS, Chicago, USA), with significance set at a p value of <0.05. A complete-case analysis approach was adopted to

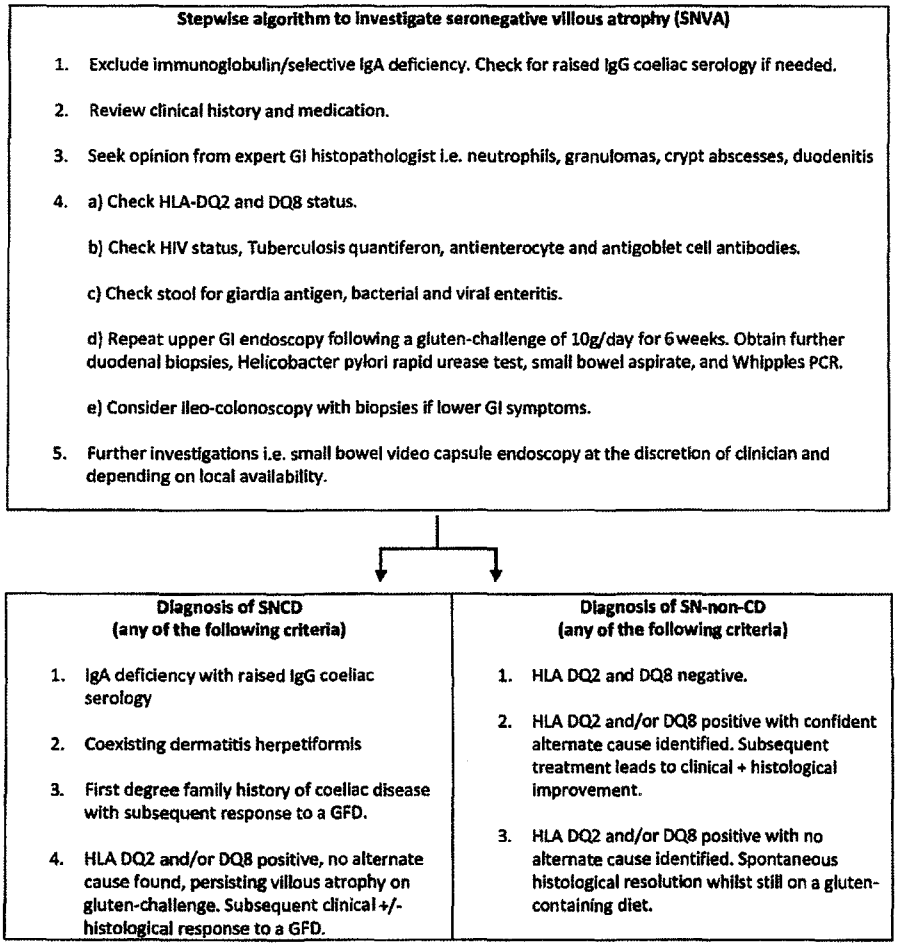

Figure 1 Stepwise proposed algorithm used to investigate and diagnose causes of seronegative villous atrophy (SNVA). GFD, gluten-free diet; HLA, human leucocyte antigen; SNCD, seronegative coeliac disease; SN-non-CD, seronegative non-coeliac disease.

address the limited data which were missing completely at random. Categorical variables were summarised by descriptive statistics, including total numbers and percentages, with comparisons between groups performed using the χ^2 test or Fisher's exact test. Normally distributed continuous variables were summarised by mean and SD with comparisons between groups performed using the unpaired Student's t-test. We performed dichotomous logistic regression between the SNCD and SN-non-CD groups using a forward stepwise method with a p value of <0.1 for entry into the analysis with all variables available for inclusion into the model. Finally, overall survival was analysed using Kaplan-Meier curves and significance compared using the log-rank test.

RESULTS

Characteristics of SNVA

The baseline characteristics of the 200 patients with SNVA are provided in table 2. The patient cohort comprised 83%

Table 2 Baseline characteristics of SNVA subjects and SPCD controls

	Total SNVA (n=200)	SN-non-CD (n=138; 69%)	SNCD (n=62; 31%)	p Value (between SNCD and SN-non-CD)	SPCD controls (n=343)	p Value (SPCD controls vs SN-non-CD)	p Value (SPCD controls vs SNCD)
Demographics							
Mean age±SD	51.2±17.6	51.4±17.3	50.9±18.2	0.89	43.5±17.3	**<0.001**	**0.002**
Female, n	127 (63.5%)	84 (60.9%)	43 (69.4%)	0.25	242 (70.6%)	**0.04**	0.85
White ethnicity, n	165 (82.5%)	105 (76.1%)	60 (96.8%)	**<0.001**	307 (89.5%)	**<0.001**	0.1
Sheffield city resident, n	166 (83%)	116 (84%)	50 (80.6%)	0.55	343 (100%)	**<0.001**	**<0.001**
Clinical symptoms							
Diarrhoea, n	120 (60%)	84 (61%)	36 (58.1%)	0.71	153 (44.6%)	**0.001**	**0.05**
Weight loss, n	71 (35.5%)	52 (37.7%)	19 (30.6%)	0.34	40 (11.7%)	**<0.001**	**<0.001**
Abdominal pain, n	98 (49%)	69 (50%)	29 (46.8%)	0.67	121 (35.3%)	**0.003**	0.08
Bloating, n	62 (31%)	43 (31.2%)	19 (30.6%)	0.94	102 (29.7%)	0.76	0.89
Dyspepsia, n	28 (14%)	24 (17.4%)	4 (6.5%)	**0.04**	19 (5.5%)	**<0.001**	0.79
Reflux, n	32 (16%)	24 (17.4%)	8 (12.9%)	0.42	27 (7.9%)	**0.002**	0.19
Nausea, n	39 (19.5%)	27 (19.6%)	12 (19.4%)	0.97	22 (6.4%)	**<0.001**	**0.001**
Constipation, n	32 (16%)	24 (17.4%)	8 (12.9%)	0.42	52 (15.2%)	0.54	0.65
Fatigue, n	32 (16%)	19 (13.8%)	13 (21%)	0.2	91 (26.5%)	**0.003**	0.36
Past medical history							
Autoimmunity, n	37 (18.5%)	18 (13%)	19 (30.6%)	**0.003**	72 (21%)	**0.04**	0.09
Family history of CD, n	7 (3.5%)	0 (0%)	7 (11.2%)	**<0.001**	55 (16%)	**<0.001**	0.34
Recent gastroenteritis-type history, n	23 (11.5%)	18 (13%)	5 (8.1%)	0.31	8 (2.3%)	**<0.001**	**0.03**
Crohn's disease, n	1 (0.5%)	1 (0.7%)	0 (0%)	0.5	1 (0.3%)	0.49	1.0
Lymphoproliferative disorders, n	4 (2%)	3 (2.2%)	1 (1.6%)	1.0	2 (0.6%)	0.15	0.39
HIV, n	2 (1%)	2 (1.4%)	0 (0%)	1.0	1 (0.3%)	0.2	1.0
Tuberculosis, n	2 (1%)	2 (1.4%)	0 (0%)	1.0	1 (0.3%)	0.2	1.0
Medication							
A2RB, n	8 (4%)	6 (4.3%)	2 (3.2%)	1.0	8 (2.3%)	0.23	0.66
Aspirin, n	29 (14.5%)	21 (15.2%)	8 (12.9%)	0.67	29 (8.5%)	**0.03**	0.26
NSAIDs, n	19 (9.5%)	16 (11.6%)	3 (4.8%)	0.13	11 (3.2%)	**<0.001**	0.46
Methotrexate, n	2 (1%)	1 (0.7%)	1 (1.6%)	0.5	2 (0.6%)	1.0	0.39
Mycophenolate, n	1 (0.5%)	1 (0.7%)	0 (0%)	1.0	1 (0.3%)	0.49	1.0
Azathioprine, n	0 (0%)	0 (0%)	0 (0%)	–	3 (0.9%)	0.56	1.0
Bloods							
Anaemia, n	61/198 (30.8%)	43/137 (31.4%)	18/61 (29.5%)	0.79	154/343 (44.9%)	**0.007**	**0.03**
Low ferritin, n	75/190 (39.5%)	51/130 (39.2%)	24/60 (40%)	0.92	207/333 (62.2%)	**<0.001**	**0.001**
Low folate, n	35/192 (18.3%)	28/131 (21.4%)	7/61 (11.5%)	0.1	100/333 (30%)	0.06	**0.003**
Low vitamin B_{12}, n	31/193 (16.1%)	21/131 (16%)	10/62 (16.1%)	0.99	61/331 (18.4%)	0.54	0.67
Low calcium, n	22/186 (11.8%)	17/126 (13.5%)	5/60 (8.3%)	0.31	28/332 (8.4%)	0.1	0.98
Low albumin, n	21/198 (10.6%)	19/137 (13.9%)	2/61 (3.3%)	**0.03**	16/336 (4.8)	**0.001**	1.0
Raised ESR and/or CRP, n	51/192 (26.6%)	40/132 (30.3%)	11/60 (18.3%)	**0.08**	82/324 (25.3%)	0.28	0.25
HLA-DQ2 and/or DQ8 positive, n	118/193 (61%)	58/133 (43.6%)	60/60 (100%)	**<0.001**	112/112 (100%)	**<0.001**	1.0
Duodenal histology							
Intraepithelial lymphocytosis, n	177 (88.5%)	116 (84%)	61 (98.4%)	**0.003**	343 (100%)	**<0.001**	0.15
Crypt hyperplasia, n	177 (89%)	117 (84.8%)	61 (98.4%)	**0.003**	343 (100%)	**<0.001**	0.15
Partial villous atrophy, n	159 (79.5%)	120 (87%)	39 (62.9%)		82 (23.9%)		
Subtotal villous atrophy, n	25 (12.5%)	10 (7.2%)	15 (24.2%)	**<0.001**	144 (42%)	**<0.001**	**<0.001**
Total villous atrophy, n	16 (8%)	8 (5.8%)	8 (12.9%)		117 (34.1%)		

Bold values are statistically significant.
A2RB, angiotensin-2-receptor-blocker; CD, coeliac disease; CRP, C reactive protein; ESR, erythrocyte sedimentation rate; HLA, human leucocyte antigen; NSAIDs, non-steroidal anti-inflammatory drugs; SNCD, seronegative coeliac disease; SN-non-CD, seronegative non-coeliac disease; SNVA, seronegative villous atrophy; SPCD, seropositive coeliac disease.

(n=166) who were residents of Sheffield and thus classed as secondary-care referrals. There were 17% (n=34) who were referred from another city for a tertiary-care opinion. The mean age was 51.2 years, with 63.5% (n=127) female and 82.5% (n=165) of white ethnicity.

The most frequently reported clinical symptoms were diarrhoea (60%, n=120), abdominal pain (49%, n=98), weight loss (35.5%, n=71) and bloating (31%, n=62). Autoimmunity was present in 18.5% (n=37) of cases, with 3.5% (n=7) also having a family history of CD. A recent history suggestive of gastroenteritis was elicited in 11.5% (n=23) of cases. The use of A2RBs was seen in 4% (n=8), of which 7 were on candesartan and 1 was on irbesartan; no patient was taking olmesartan.

Blood tests revealed anaemia in 30.8%, with associated haematinic deficiencies ranging from 16.1% to 39.5%. A raised erythrocyte sedimentation rate and/or C reactive protein was present in 26.6% of patients. The presence of positive HLA-DQ2 and/or DQ8 was seen in 61.1% (n=118/193).

Finally, histological grading of duodenal biopsies showed intraepithelial lymphocytosis in 88.5% (n=177), with the majority of patients found to have PVA at 79.5% (n=159). In contrast, SVA was seen in 12.5% (n=25) and TVA in 8% (n=16).

Aetiology of SNVA

Following systematic evaluation of 200 SNVA cases, we diagnosed SNCD in 31% (n=62) of cases with the remaining 69% (n=138) due to SN-non-CD. The breakdown of all causes is shown in figure 2.

In the 62 cases identified as having SNCD, 14 were diagnosed with relative ease based on (1) selective IgA deficiency but with raised IgG coeliac serology (n=9, three also had associated first degree family history of CD), (2) first degree family history of CD alone with subsequent response to a GFD (n=4) and (3) dermatitis herpetiformis (n=1). The other 48 patients were diagnosed with SNCD on the basis of having positive HLA-DQ2 and/or DQ8 status, no alternate cause found, persisting villous atrophy following a gluten rechallenge, with subsequent clinical±histological response to a GFD.

A wide range of aetiologies was established in the 138 SN-non-CD cases, commonly infective, medication-induced and inflammatory in nature. In total, there were 54 cases attributed to an infection. This included *Helicobacter pylori* induced duodenitis alone (n=21) or in conjunction with *Mycobacterium tuberculosis* (n=2), Mycobacterium avium intracellulare (n=1) and HIV (n=1). Other causes included viral gastroenteritis based upon clinical history (n=7), giardiasis (n=6), small bowel bacterial overgrowth (n=4), HIV enteropathy (n=2), ascariasis (n=2), Mycobacterium tuberculosis (n=2), tropical sprue (n=2), Campylobacter (n=1), candidiasis (n=1), Whipple's disease (n=1) and Mycobacterium avium intracellulare (n=1). There were 13 cases which occurred as a result of medication; 9 due to non-steroidal anti-inflammatory drug-related duodenitis, and the others were a case each related to methotrexate, mycophenolate mofetil, irbesartan and a possible association with candesartan. There were 23 cases of non-specific peptic duodenitis, 6 cases of Crohn's disease and 4 cases due to systemic immune-mediated disorders which included a case each of sarcoidosis, graft versus host disease, autoimmune enteropathy and common variable immunodeficiency. There was a case each of radiation enteritis and eosinophilic enteritis. Following appropriate treatment these showed clinical and histological improvement.

Finally, in 36 cases of SN-non-CD, despite extensive investigations, we were unable to elicit any cause and these patients were labelled as idiopathic/unclassified sprue. Interestingly, 72% (n=26/36, 11 of whom were HLA-DQ2/8 positive) had spontaneously normalised their duodenal biopsies when rechallenged with gluten, suggesting transient villous atrophy. This was seen on average 9 months after the index biopsy had shown villous atrophy. Of the remaining 10 cases, all HLA-DQ2/8 negative, 4 required immunosuppressive therapy for persisting unexplained villous atrophy with the other 6 either lost to follow-up or refusing further endoscopic investigations given their clinical stability.

Risk factors for diagnostic outcomes

Univariate analysis comparing the SNVA subgroups and SPCD controls are shown in table 2. In summary, the SNVA cohort was older at the time of presentation and more likely to present with symptoms of diarrhoea, abdominal pain, nausea and weight loss.

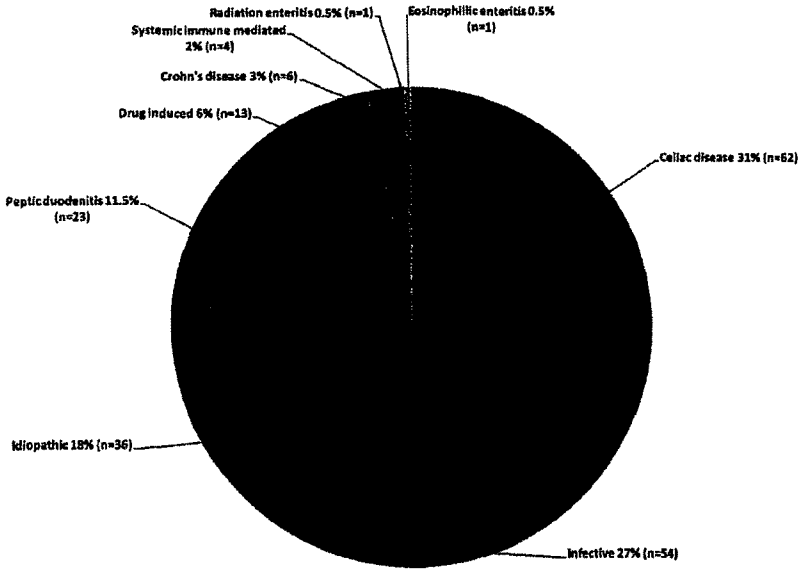

Figure 2 Causes of seronegative villous atrophy (SNVA) at a UK centre (n=200).

In contrast, subjects with SPCD or SNCD were more likely than SN-non-CD to have autoimmunity, family history, and HLA-DQ positivity; however, the positive predictive value of HLA-DQ2/8 for SNCD in the context of SNVA was only 51% (n=60/118). There was also a significant trend towards lesser degrees of villous atrophy from SPCD towards SNCD and then SN-non-CD.

Factors significantly associated with SN-non-CD included non-white ethnicity, dyspepsia, negative HLA-DQ status, lack of intraepithelial lymphocytosis/crypt hyperplasia and hypoalbuminaemia. Multivariable logistic regression analysis of the SNVA cohort showed that an independent factor associated with a diagnosis of SN-non-CD was non-white ethnicity (OR 10.8, 95% CI 2.2 to 52.8, p=0.003). Indeed, 23 of the 35 (66%) non-white subjects presenting with SNVA had a GI infection, commonly *H. pylori* induced duodenitis; table 3. Only 2 of 35 (5.7%) non-whites with SNVA had SNCD compared with 60 of 165 (36%) whites.

Immunophenotyping of intraepithelial lymphocytes

Immunohistochemistry was performed in 19 SNVA cases of which 14 were SN-non-CD and 5 SNCD. Both groups showed CD8-positive T cytotoxic intraepithelial lymphocytes, similar to that seen in SPCD controls. However, four cases of SN-non-CD also contained CD4-positive T helper cells among the intraepithelial lymphocytes; these cells are associated with refractory CD within the context of CD but in other contexts, such as GI infection, they are a normal component of the immune response (see figure 3 and online supplementary figure S1).

Table 3 Characteristics and diagnostic outcomes in non-whites with SNVA seen at a UK centre

Case	Age/sex	Ethnicity	Symptoms	Marsh grading	HLA-DQ2/8 status	Aetiology of SNVA
1	28/female	Pakistan	Abdominal pain, reflux, weight loss	PVA	+	*H. pylori* induced duodenitis
2	29/male	Oman	Diarrhoea, nausea, bloating, dyspepsia	PVA	+	No cause found—SNVA resolved
3	30/female	Tunisia	Diarrhoea, anaemia, bloating	PVA	+	Giardiasis
4	33/male	Ghana	Diarrhoea, weight loss, abdominal pain, cough	PVA	+	Sarcoidosis
5	39/male	Somalia	Diarrhoea, bloating, abdominal pain	PVA	+	Small intestinal bacterial overgrowth
6	43/male	Iran	Nausea, dyspepsia, cough, weight loss	PVA	+	Tuberculosis
7	49/female	Pakistan	Anaemia, abdominal pain, bloating, reflux, fatigue, constipation	PVA	+	Small intestinal bacterial overgrowth
8	49/female	India	Anaemia	PVA	+	*H. pylori* induced duodenitis
9	49/female	Somalia	Diarrhoea, bloating, abdominal pain, anaemia	PVA	+	No cause found—SNVA resolved
10	58/female	India	Abdominal pain, dyspepsia, bloating	PVA	+	No cause found—SNVA resolved
11	65/female	Iraq	Abdominal pain, bloating, dyspepsia, anaemia	PVA	+	Whipple's disease
12	82/female	Yemen	Abdominal pain, bloating, reflux	PVA	+	Peptic duodenitis
13	18/male	Pakistan	Anaemia	SVA	+	Coeliac disease. Associated Sjogren's and IgA deficiency
14	31/female	Pakistan	Diarrhoea, anaemia, nausea, bloating	SVA	+	Mycobacterium avium, *H. pylori* induced duodenitis
15	53/female	India	Anaemia	SVA	+	NSAIDs
16	71/male	Bangladesh	Anaemia, dyspepsia, bloating	SVA	+	*H. pylori* induced duodenitis
17	30/male	Iran	Dyspepsia, reflux, weight loss	SVA	+	Coeliac disease
18	22/female	Pakistan	Abdominal pain, nausea, fatigue	PVA	–	*H. pylori* induced duodenitis
19	26/female	Yemen	Anaemia, weight loss, abdominal pain. nausea, bloating, fatigue	PVA	–	*H. pylori* induced duodenitis
20	35/female	Caribbean	Diarrhoea, bloating, abdominal pain	PVA	–	*H. pylori* induced duodenitis
21	36/male	Iraq	Diarrhoea, abdominal pain, bloating	PVA	–	NSAIDs
22	38/male	Zambia	Anaemia, abdominal pain	PVA	–	Tuberculosis, *H. pylori* induced duodenitis
23	38/female	Bangladesh	Diarrhoea, anaemia, abdominal pain, fatigue	PVA	–	*H. pylori* induced duodenitis
24	45/female	Pakistan	Anaemia	PVA	–	*H. pylori* induced duodenitis
25	47/female	Vietnam	Dyspepsia	PVA	–	No cause found—lost to follow-up
26	47/male	Pakistan	Abdominal pain, weight loss, bloating, reflux	PVA	–	Peptic duodenitis
27	47/female	Pakistan	Diarrhoea, bloating	PVA	–	Ascariasis
28	49/female	Bangladesh	Diarrhoea, anaemia, fatigue, fevers, night sweats	PVA	–	Tuberculosis
29	50/male	Caribbean	Diarrhoea, abdominal pain, reflux	PVA	–	*H. pylori* induced duodenitis
30	51/female	Yemen	Abdominal pain, weight loss	PVA	–	*H. pylori* induced duodenitis
31	54/female	Bangladesh	Diarrhoea, weight loss, abdominal pain, dyspepsia, fatigue, constipation	PVA	–	No cause found—SNVA resolved
32	75/female	Hong Kong	Diarrhoea, weight loss, anaemia	PVA	–	Small intestinal bacterial overgrowth
33	35/male	Bangladesh	Reflux, dyspepsia, weight loss	SVA	–	*H. pylori* induced duodenitis
34	57/male	Yemen	Diarrhoea, weight loss	PVA	Not stated	*H. pylori* induced duodenitis
35	25/male	Caribbean	Diarrhoea	SVA	Not stated	HIV enteropathy, *H. pylori* induced duodenitis

HLA, human leucocyte antigen; NSAIDs, non-steroidal anti-inflammatory drugs; PVA, partial villous atrophy; SNVA, seronegative villous atrophy; SVA, subtotal villous atrophy.

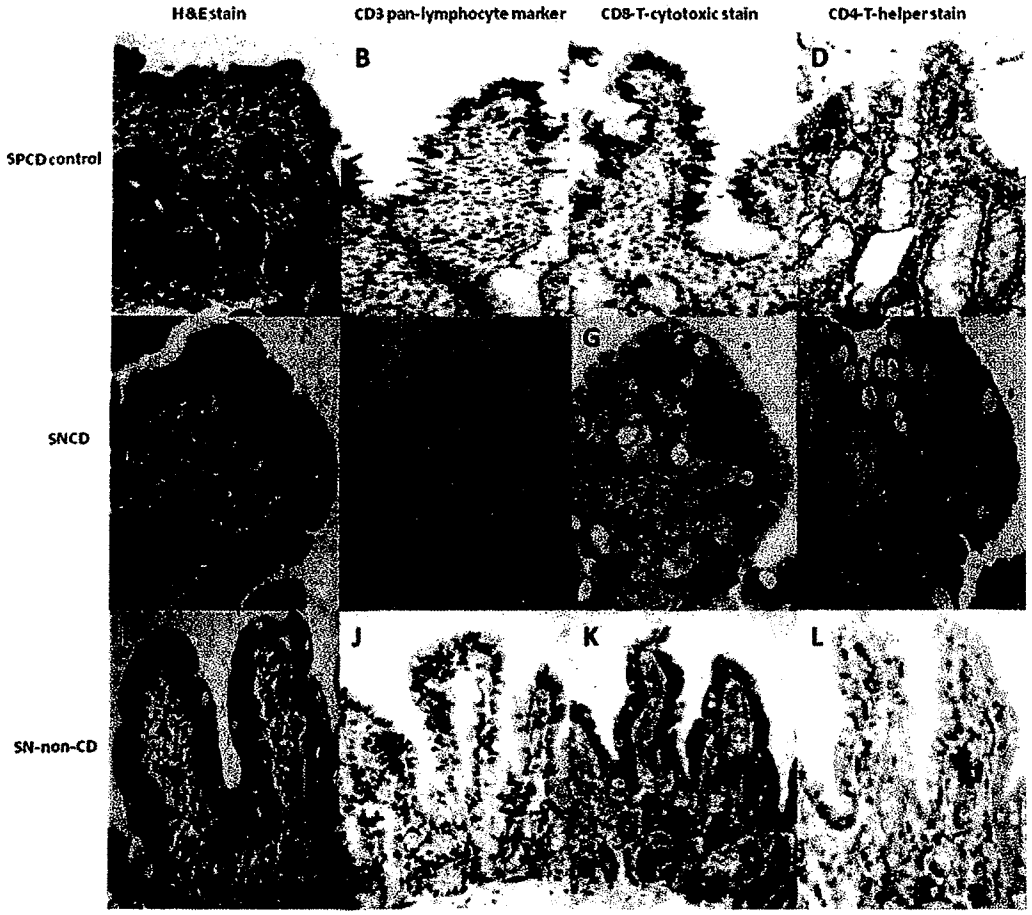

Figure 3 Plates A to D: SPCD control. A white female presenting with anaemia and positive serum IgA endomysial antibody. Duodenal biopsy demonstrated subtotal villous atrophy when stained with haemotoxylin and eosin (H&E). It can also be seen that there is an increased number of intra-epithelial lymphocytes stained by the pan-lymphocyte marker CD3. Furthermore, staining of antibodies against different intraepithelial lymphocyte phenotype revealed that they are all of the CD8-T-cytotoxic stain and not CD4-T-helper cells. This is the classical pattern of coeliac disease. Plates E to H: SNCD patient. A white female presenting with diarrhoea. Serum IgA endomysial antibodies were negative but duodenal biopsy showed subtotal villous atrophy. There were increased intraepithelial lymphocytes noted following CD3 pan-lymphocyte stain, which on immunophenotypic differentiation revealed CD8-T-cytotoxic cells but not CD4-T-helper cells. Her HLA-DQ2 was positive, no alternate cause was found, and she responded to a gluten-free diet. Plates I to L: SN-non-CD patient. Bengali female presenting with diarrhoea, anaemia, night sweats and fevers. Her serum IgA endomysial antibody was negative but duodenal biopsy showed partial villous atrophy with raised intraepithelial lymphocytes. She stained positive for CD8-T-cytotoxic cells but also for CD4-T-helper cells. This could have been mistaken for refractory coeliac disease. However, her HLA-DQ2/8 genotype was negative and on microbiology assessment her duodenal sample revealed mycobacteria (supplementary Figure S1). She was commenced on anti-tuberculosis therapy. Duodenal histology of seronegative villous atrophy (SNVA) and seropositive coeliac disease (SPCD) control. HLA, human leucocyte antigen; SNCD, seronegative coeliac disease; SN-non-CD, seronegative non-coeliac disease.

Survival analysis

There have been 19 deaths within the 200 SNVA cohort, of which 7/60 (11.2%) were in the SNCD group and 12/138 (8.7%) in the SN-non-CD group. In comparison there have been 11/343 (3.2%) deaths in the SPCD controls. On Kaplan-Meier analysis there were no statistical differences in estimated survival between the SNVA groups although this was less favourable compared with SPCD (figure 4: log-rank p=0.002).

DISCUSSION

Main findings

We believe that our findings represent a major conceptual change in the understanding and management of SNVA. Having used a systematic clinical algorithm we have shown that SNCD accounts for 31% of SNVA cases, with the remaining 69% due to SN-non-CD related causes. Furthermore, HLA-DQ2 and/or DQ8 genotype was present in 61% of SNVA cases, with a positive predictive value of only 51% for a diagnosis of SNCD. This is not surprising given that these alleles are common as seen in approximately 40% of the general population.[3]

Importantly, we have identified that non-white ethnicity is a risk factor to alert clinicians to the possibility of SN-non-CD, in particular with regards to an infective aetiology. These findings are the first to be reported outside of the tropics and in a Western society.[25] The clinical relevance of this also expands to the USA where results from a national pathology database have identified that among patients undergoing duodenal biopsies it

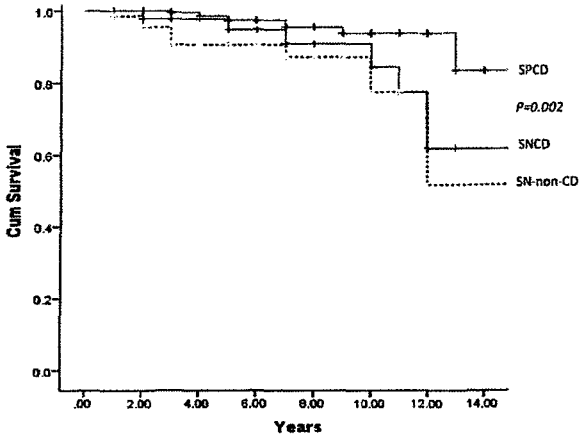

Figure 4 Kaplan-Meier estimated survival curves for seronegative villous atrophy (SNVA) and seropositive coeliac disease (SPCD) controls. SNCD, seronegative coeliac disease; SN-non-CD, seronegative non-coeliac disease.

is those from the Punjab region of India that constitute the ethnic group with the highest prevalence of villous atrophy.[52] It remains to be determined whether such patients had SNVA given that the US National Health and Nutrition Examination survey has found positive coeliac serology to be rare among non-whites.[1]

In addition, in almost one in five cases of SNVA no identifiable cause was found although, reassuringly, the majority spontaneously normalised duodenal histology while being investigated on a gluten-enriched diet; had these patients been commenced on a GFD at the outset instead, they would have erroneously been diagnosed with SNCD and wrongfully subjected to a lifelong, restrictive diet. This, along with previous studies showing an empirical trial of a GFD to be a poor predictor of CD,[17] further supports the notion that clinicians must not start a GFD in SNVA until investigations are complete and a firm diagnosis of SNCD has been established.

Finally, differences in survival outcomes between SNVA and SPCD controls were noted. A recent English study involving more than 10 000 patients with CD found no major excess risk of cancer, digestive disease or respiratory disease related or cardiovascular mortality compared with the general population.[53] However, it is recognised that those with SNCD tend to be older and run a more advanced disease course than SPCD.[15] With regards to SN-non-CD this entity has a number of heterogeneous disease associations (ie, HIV, tuberculosis, common variable immunodeficiency) which are associated with poorer outcomes.

Strengths and limitations

The main strength of this study is that it is the largest and most comprehensive to date, having prospectively evaluated 200 consecutive adult patients with SNVA at a UK secondary/tertiary-care centre over a 15-year period. The cohort studied included both inner and outer city referrals. Moreover, systematic and rigorous investigations were performed using testing modalities available among most gastroenterology departments. We therefore feel that our findings can be used as a benchmark and generalised to other physicians seeing similar patients.

However, our study does have several limitations. First, we do not perform serum deamidated gliadin peptide antibodies or intestinal coeliac antibody deposits, both of which are relatively novel markers and can aid towards the diagnosis of CD.[54–56] Second, it may also be perceived that by identifying and including IgA deficient patients who were subsequently found to be IgG coeliac serology positive (n=9) is a weakness in that this should be common knowledge. However, our findings are those of real life practice and would be supported by other groups who have shown that inadequate evaluation of IgA deficiency occurs frequently when testing for CD.[57] Nevertheless, had we excluded such patients from our analysis then the prevalence of SNCD would have been 27.7% (n=53/191) instead of the 31% (n=62/200) stated. Third, we have unanswered questions in those in whom no cause was found (so called idiopathic/unclassified sprue) but spontaneously normalised duodenal biopsies while consuming high-dose gluten. A recent case series has highlighted that self-limiting enteropathies can occur in the context of GI infections,[23] which raises the possibility that our patients may have experienced a similar insult although this was not recalled from their clinical history nor isolated from stool cultures. Furthermore, these individuals had their repeat biopsy performed on average 9 months after the index case which had shown villous atrophy. We do not know when their histology started improving and at what exact time point it had normalised. Had the biopsies been performed earlier then these patients may still have had persisting villous atrophy and, in those with the correct HLA-DQ genotype, subsequently categorised as having CD. However, our study was performed pragmatically and is a reflection of routine outpatient clinical practice. Nevertheless, future research studies should aim to perform biopsies at sequential time points. Finally, of those carrying the HLA-DQ genotype it could be hypothesised that these individuals may still belong to the spectrum of CD and have simply experienced an unexplained GI insult transiently manifesting as SNVA but having not yet reached the cumulative threshold required for CD to become apparent.[58] Hence, longitudinal follow-up data are now required in this particular group.

Other studies

To our knowledge only one other study has evaluated diagnostic outcomes in SNVA.[16] This was performed by the New York group who evaluated 72 complex case referrals of SNVA over a 10-year period. The investigators found that 22% (n=16/72) of their SNVA cases were due to olmesartan-related enteropathy.[16] This novel association has generated substantial interest and is of importance given its presentation may be that of a severe form of enteropathy necessitating hospitalisation for the management of intractable diarrhoea, weight loss, dehydration, hypotension, acute renal failure and metabolic acidosis.[16 42–45] Yet, these findings are in contrast to ours where the use of A2RB was seen in 8 of 200 SNVA cases, with A2RB a responsible cause for enteropathy in two patients; overall prevalence of A2RB enteropathy being 1% (n=2/200). In the other six patients we found an alternate aetiology for SNVA with patients well maintained on their A2RB; these include CD (n=2), giardiasis (n=1), eosinophilic enteritis (n=1), small bowel bacterial overgrowth (n=1) and loss to follow-up (n=1). Given that A2RBs, including olmesartan, are dispensed in the UK, the discrepancy in the results raises two main points. First, the high prevalence of olmesartan-related enteropathy reported elsewhere may not be reflecting SNVA in general but rather groups overseeing and presenting the outcomes of cases referred from wide catchment areas with presumed 'poorly responsive/refractory CD'.[16] In fact, the initial case series highlighting this association came from the Mayo Clinic where 22 patients with olmesartan-related enteropathy were reported following

referrals from 16 US states over a 3-year period.[42] Following on, a nationwide multicentre French survey identified 36 patients with olmesartan-related enteropathy.[44] Most recently, the crude incidence rates of olmesartan and other A2RB enteropathy in France has been calculated at 5.6 and 1.8 per 100 000 patient years, respectively.[45] These findings suggest that olmesartan-related and in particular other A2RB-related enteropathies are rare adverse events. Second, despite the growing awareness of A2RB-related enteropathy clinicians must still remain vigilant that on occasions A2RBs will merely be innocent bystanders and an alternate aetiology for SNVA will be found.

CONCLUSION

This large UK centre study provides a prospective, systematic and clinically pragmatic evaluation of SNVA. We have shown that most patients with SNVA do not have CD or A2RB enteropathy. Further, a subgroup in whom no cause is found will show spontaneous histological resolution while still consuming gluten and this phenomenon requires further evaluation. The presence of non-white ethnicity was found to be a factor predicting a non-coeliac cause, in particular infective aetiology.

Contributors IA designed the study, recruited patients, collected data, performed statistical analysis, wrote and edited the manuscript. MFP, J-HB, VK, JCW, DP, PV collected data. SSC collected data, performed statistical analysis, and edited the manuscript. PHG edited the manuscript for important intellectual content. DSS conceived and designed the study, recruited patients, collected data, and edited the manuscript. All authors reviewed and approved the final version of the manuscript.

Competing interests None declared.

Ethics approval The initial ethics was from the South Sheffield Research and Ethics Committee, then the Humber Research and Ethics Committee 09/H1304/69. The study was also registered under Sheffield Teaching Hospitals audit number 2954.

Provenance and peer review Not commissioned; externally peer reviewed.

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GUT

The clinical and phenotypical assessment of seronegative villous atrophy; a prospective UK centre experience evaluating 200 adult cases over a 15-year period (2000–2015)

Imran Aziz, Mohammad F Peerally, Jodie-Hannah Barnes, Vigneswaran Kandasamy, Jack C Whiteley, David Partridge, Patricia Vergani, Simon S Cross, Peter H Green and David S Sanders

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Exhibit G

AP&T Alimentary Pharmacology and Therapeutics

Systematic review: sprue-like enteropathy associated with olmesartan

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SUMMARY

Background
The onset of a sprue-like enteropathy in association with olmesartan therapy has been recently reported.

Aims
To perform a systematic review of the literature and describe three additional cases of olmesartan-associated enteropathy.

Methods
Electronic and manual bibliographic searches were performed to identify original reports in which subjects who were undertaking olmesartan developed a sprue-like enteropathy. Because of the scarcity of studies with adequate sample size, case series with less than 10 patients and case reports were also considered. Data extraction was performed independently by two reviewers.

Results
A total of 11 publications met our pre-defined inclusion criteria, for an overall number of 54 patients (including our series). Almost all patients presented with diarrhoea and weight loss. Normocytic normochromic anaemia and hypoalbuminaemia were the commonest laboratory defects at presentation. Antibody testing for coeliac disease was always negative. Variable degrees of duodenal villous atrophy were present in 98% of patients, while increased intra-epithelial lymphocytes were documented in only 65% of cases. After discontinuation of olmesartan, all reported patients achieved resolution of signs and symptoms.

Conclusions
Although the available evidence is limited, the olmesartan-associated sprue-like enteropathy may be considered as a distinct clinical entity, and should be included in the differential diagnosis when serological testing for coeliac disease is negative.

Aliment Pharmacol Ther 2014; 40: 16–23

doi:10.1111/apt.12780

INTRODUCTION

Coeliac disease is the most common cause of villous atrophy and increase in intra-epithelial lymphocytes (IELs) in the small bowel.[1] However, these histopathological findings are also caused by other disorders, such as Crohn's disease, enteric infections (e.g. *Giardia lamblia*), collagenous sprue, tropical sprue, common variable immunodeficiency, autoimmune enteropathy and haematological malignancies.[2] Villous atrophy and malabsorption have been reported also as a side-effect of immunosuppressant drugs (in particular, azathioprine, methotrexate, neomycin and mycophenolate mofetil).[3–6]

Olmesartan medoxomil is an angiotensin II receptor blocker used for the management of hypertension, available in Western Countries since 2002.[7] A sprue-like enteropathy associated with olmesartan has been first described in 2012 by Rubio-Tapia *et al.*,[8] and similar cases have since been reported. To date, the pathogenic mechanism of olmesartan-associated enteropathy is still unknown.

Here, we have performed a systematic review of sprue-like enteropathy occurring during treatment with olmesartan and also report three additional cases. Furthermore, we assessed all published reports through a systematic review of literature.

Case series

Case #1. A 60-year old man presented with weight loss and nonbloody diarrhoea. Apart from arterial hypertension, treated with olmesartan since 3 years, his medical history was unremarkable. Physical examination was normal, as well as routine blood and stool exams. Total type A immunoglobulins (IgA) were normal. Both antibody testing for coeliac disease (e.g. transglutaminase and endomisium antibodies) and DQ2/DQ8 HLA search were negative. Colonoscopy findings were normal. Upper endoscopy showed a nodular appearance of the mucosa and a complete flattening of villi. Histopathological examination confirmed the absence of any villous pattern, but without increase in IELs. Neither gluten-free diet nor steroid administration improved symptoms. After reading the report by Rubio-Tapia *et al.*,[8] we hypothesised the possible causative role of olmesartan in the development of such findings. Olmesartan was therefore withdrawn and replaced with ramipril. One week after, diarrhoea ceased and the patient began to gain weight. At 3-month follow-up, full recovery of duodenal villi was documented both at endoscopic assessment and at histopathological analysis.

Case #2. A 59-year old man was admitted to our hospital because of severe fatigue, weight loss and diarrhoea. He was respectively taking insulin because of diabetes mellitus type II and olmesartan because of arterial hypertension. Abdomen physical examination was normal, as well as fasting blood sugar. Laboratory evaluation revealed hypochromic microcytic anaemia and hypoalbuminaemia. Stool exams were negative. Suspecting a neoplastic aetiology, we performed a colonoscopy (that was unremarkable) and an upper endoscopy that showed a severe hypotrophy of duodenal villous pattern. The histological assessment documented a partial villous atrophy, without increase in IELs. Patient therefore underwent full laboratory testing for coeliac disease, including transglutaminase and endomisium antibodies, total blood IgA, human leucocyte antigen (HLA) assessment, without any relevant finding. Anyway, a gluten-free diet was started, without clinical success. We therefore attempted to switch from olmesartan to amlodipine. Afterwards, the patient experienced first disappearance of diarrhoea and then weight gain. The 3-month follow-up upper endoscopy showed the growth of duodenal villi that was confirmed at histopathological evaluation.

Case #3. A 78-year old woman was hospitalised because of fatigue, dyspnoea and nonbloody diarrhoea. She had formerly been diagnosed with coeliac disease, because of an histological finding of total villous atrophy and increase in IELs, even though testing for anti-transglutaminase and endomysial antibodies and typing for HLA DQ2/DQ8 were negative. Since 2010, the patient was receiving a gluten-free diet, without any further follow-up. She was also on olmesartan and furosemide because of arterial hypertension. Physical examination revealed a diffused body oedema, with ascites and pleural effusion. Laboratory assessment revealed normochromic normocytic anaemia and severe hypoalbuminaemia. Total IgA, anti-endomysial and transglutaminase antibodies were within normal range. Cardiac and liver ultrasonography were unremarkable, and a CT scan confirmed the presence of diffuse tissue oedema. At upper endoscopy, total atrophy of duodenal villi was observed, together with a nodular mucosal pattern. Histological assessment confirmed total villous atrophy, but without increase in IELs (Figure 1a). In the suspicion of olmesartan-associated sprue-like enteropathy previously misdiagnosed as seronegative coeliac disease, we changed olmesartan to barnidipine. After 2 weeks, diarrhoea disappeared and anasarca improved markedly. After 2 months,

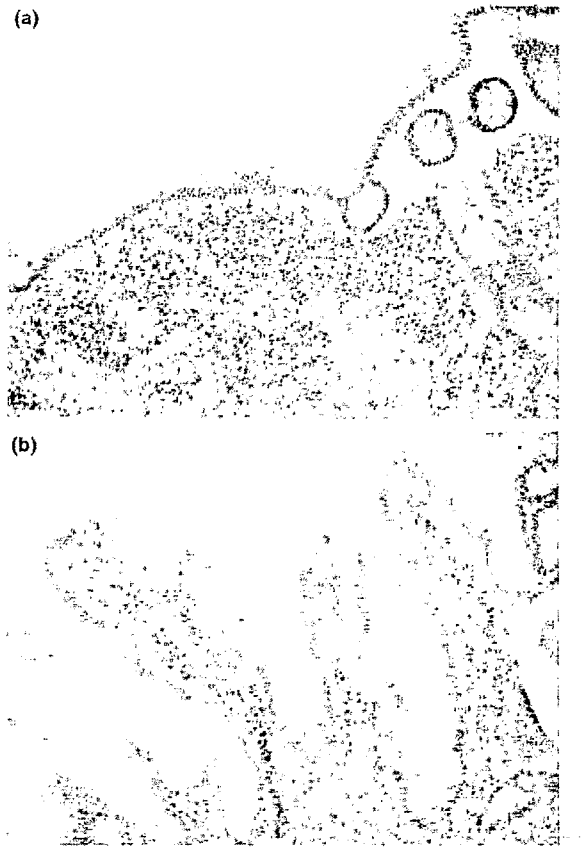

Figure 1 | Histology before (a) and after (b) suspension of olmesartan in case 3 (haematoxylin-eosin, original magnification 200×). In (a), complete absence of duodenal villi without increasing of IELs; in (b), partial reconstitution of villi.

anaemia improved and serum albumin returned into normal range. Tissue oedema had disappeared almost completely. Moreover, follow-up endoscopy showed a patchy partial recovery of duodenal villi; histological analysis confirmed the presence of partial villous atrophy (Figure 1b).

METHODS

This systematic review was conducted, when possible, in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines.[9]

Eligibility criteria

All the original reports in which the development of sprue-like enteropathy was documented in patients of any age being treated with olmesartan were considered for inclusion. Studies evaluating treatments other than olmesartan were excluded, as well as those including patients without enteropathy treated with olmesartan. In the case of mixed cohorts, only data from patients treated with olmesartan were taken into account. We did not include animal model studies or non-original reports. Because of the likely scarcity of studies with adequate sample size, also case series with less than 10 patients and case reports were considered, without year- span limits. No language restriction was used in the search filter. We included also data presented only as abstracts at conferences.

Information sources and search strategy

A literature search was performed using the following electronic databases: PubMed, SCOPUS, Web of Science (ISI), the Cochrane Library. The last search was run on 19 February 2014. The term 'olmesartan' was matched with the following words: 'villous' OR 'villar' OR 'villous atrophy' OR 'villar atrophy' OR 'enteropathy' OR 'sprue-like' OR 'spruelike' OR 'celiac' OR 'coeliac' OR 'sprue' OR 'diarrhea' OR 'diarrhoea'. All the terms were searched both as keywords and Medical Subject Headings (MeSH). The bibliographies of relevant (according to titles and abstracts) articles were hand-searched to provide additional references. Records from the following yearly symposia were hand-searched to find pertinent abstracts: United European Gastroenterology), 2008–2013; Digestive Disease Week, 2001–2013; European Crohn's and Colitis Organization Congress, 2007–2013; Crohn's and Colitis Foundation of America Annual Scientific Meeting, 2003–2013. When necessary, authors of articles were also contacted for clarifications or for missing information about their data.

Study selection

Titles and abstract were independently assessed from two reviewers (G.I. and S.B.) to determine the eligibility of the studies. Both investigators checked the fulfilment of inclusion and exclusion criteria; in the case of doubt, the full text of articles was retrieved and reviewed. A third author (G.C.) arbitrated in all the cases of a lack of agreement.

Data collection process

Data from eligible studies were independently extracted by two reviewers (G.I. and S.B.), then cross-checked. Discrepancies were rectified by consensus. When articles grouped patients from a previous study and newly enrolled ones, only the latter were considered. In the case of mixed cohorts, only data regarding patients treated with olmesartan were included for the analysis.

Data extracted from each primary study are shown in Table 1. Study references and citations were collected in Endnote software application version 6.0 (Thomson Reuters, New York, NY, USA). A data collection form was designed in Microsoft Excel 2007 (Microsoft, Redmond, WA, USA).

RESULTS

Characteristics of included studies

After literature search and review of titles and abstracts, 11 articles met our pre-defined inclusion criteria.[2, 8, 10–18] All of them were Western case series or case reports published between 2012 and 2014. All but one[10] have been published as full-text articles. A list of the excluded studies and reasons for exclusion, as well as the flow diagram of study selection, are available from the corresponding author upon request. Data from our case series were also considered for the final analysis. Therefore, a total of 12 articles (including the present article) have been included for analysis. Table 1 summarises findings from all studies reporting the onset of an olmesartan-associated enteropathy.

Characteristics of patients

A total of 54 patients, equally distributed between both genders (27 males; 27 females) developed a sprue-like enteropathy during treatment with olmesartan. The mean age was 69 (range: 47–87). The mean duration of olmesartan therapy was reported in 5[8, 10, 11, 14, 15] of 13 studies (including our series), ranging between 6 months and 7 years.

Clinical presentation

Almost all patients whose symptoms had been reported presented with diarrhoea (36 of 38 patients) (95%) and weight loss (34 of 38 patients) (89%). Other common symptoms were fatigue (21 of 38) (56%), nausea and vomiting (17 of 38) (45%), abdominal pain (14 of 38) (37); bloating (11 of 38) (29%). Less frequent symptoms were reflux symptoms, loss of appetite, mild constipation.

Laboratory evaluation

Normocytic normochromic anaemia (17 of 38 individuals) (45%) and hypoalbuminaemia (15 of 34) (39%) were the most diffused laboratory defects at presentation. Only one patient from our series showed microcytic hypochromic anaemia. When performed, the HLA assessment found presence of DQ2 or DQ8 haplotypes in 33 of 46 patients. Anti-transglutaminase and -endomysial antibodies were respectively searched in 49 and 33 patients, and were both always negative. Enterocyte antibodies were tested in 21 patients, resulting positive (with atypical pattern) in three cases.

Endoscopic appearance

The majority of included articles did not describe the endoscopic pattern of olmesartan-associated sprue-like enteropathy. Upper endoscopy showed nodularity in the duodenal bulb in one patient,[11] and duodenal ulcers in another one[14] while Stanich *et al.* did not find changes in the duodenal pattern.[15] In our series, all endoscopic exams were performed with an high-definition scope (EPK-I scope, Pentax Medical, Tokyo, Japan), using both i-scan technology and water-immersion technique for the assessment of the duodenal villous pattern and driving of biopsy sampling. Two of three patients showed a nodular appearance of the mucosa and marked villous atrophy. In the remaining patient, a partial flattening of duodenal villi was found.

Histopathological findings

Flattening of the duodenal villous pattern was the most common histopathological finding, being observed in 53 of 54 (98%) patients. Respectively, total villous atrophy occurred in 28 patients and partial villous atrophy in 22 patients (52% and 41%, respectively). In three patients,[2] the degree of villous flattening was not assessed. In one patient,[18] no villous atrophy was observed. Increased duodenal IELs, according to the modified Marsh's classification modified by Oberhuber (>40 IELs/100 enterocytes),[19] were found in 34 of 52 (65%) patients. All patients from our series showed normal duodenal IELs. In addition, a thickened subepithelial collagen layer, as occurs in collagenous sprue, was identified in the duodenum of 18 of 54 (33%) patients.

Clinical and histopathological outcomes

In all but four[11, 15–17] studies, patients were administered a gluten-free diet after being misdiagnosed as coeliac disease. Gluten withdrawal did not improve symptoms in 45 of 46 (98%) patients, being successful in only one case.[2] Steroid administration was attempted in 20 patients, leading to amelioration of symptoms in 19 (95%) of them; one patient from our series did not respond to steroid treatment.

After discontinuation of olmesartan, 100% of patients achieved resolution of diarrhoea (clinical response). Weight changes were reported in only three studies,[8, 14, 16] plus in our series: a total of 21 of 27 (78%) patients experienced weight gain after stopping olmesartan. Twenty-seven patients underwent a follow-up upper

Table 1 | Studies reporting patients with olmesartan-associated sprue-like enteropathy

Author (Reference)	*n* (M)	Age (yr) or mean age with range	Years of olmesartan intake or mean with range	Symptoms and laboratory findings (*n*)	HLA DQ2 or DQ8 positive (*n*)	TTG/EMA positive (*n*)	Anti-enterocyte antibodies positive (*n*)	Endoscopic findings (*n*)	Duodenal histology at diagnosis (*n*)	Amelioration of symptoms after after GFD (*n*)	Amelioration after steroid administration (*n*)	Clinical amelioration/ histological recovery after suspension of olmesartan (*n*)
de Fonseka[10]	1 (1)	60	7	Diarrhoea Weight loss Anaemia norm. Hypoalbuminaemia	0	0/0	NT	NR	PVA IELs	0	0	1/NR
Rubio-Tapia *et al.*[9]	22 (9)	69 (47–81)	3 (0.5–7)	Diarrhoea (22) Weight loss (22) Anaemia norm (14) Hypoalbuminaemia (10) Nausea and vomiting: 15/22. Abdominal pain: 11/22 Bloating: 9/22 Fatigue: 15/220)	17 of 21 tested	0/0 of 9 tested	3 of 19 tested	NR	TVA in 15 PVA in 7 SCD in 7 IELs in 13 (9 TVA and 4 PVA)	0	NR	22/17 of 18 tested
Talbot [11]	1 (1)	59	3	Anaemia norm.	1	0/NT	NT	Nodularity in the duodenal bulb	PVA IELs	NT	NT	NA
De Gaetani *et al.*[7]	16 (8)	67 (52–83)	NR	NR	14 of 15 tested	0/0	NT	NR	TVA in 8 PVA in 5 NSDVA in 3 SCD in 11 IELs in 11 (7TVA, 2PVA, 2 NSDVA)	1	16	15/2 of 2 tested
Dreifuss *et al.*[12]	1 (1)	64	NR	Diarrhoea Weight loss Bloating	0	0/0	NT	NR	PVA IELs	0	NT	1/NR
Nielsen *et al.*[13]	1 (0)	62	NR	Diarrhoea Weight loss Nausea and vomiting Abdominal pain Bloating	1	0/0	0	NR	TVA SCD IELs	0	NT	1/1
Nunge *et al.*[14]	1 (0)	81	NR	Diarrhoea Weight loss Hypoalbuminaemia	1	0/0	0	Duodenal ulcers	TVA IELs	0	1	1/NR
Stanich *et al.*[15]	1 (0)	57	NR	Diarrhoea Hypoalbuminaemia Nausea and vomiting Abdominal pain	1	0/0	NT	Normal duodenal pattern	TVA IELs	NT	NT	1/NR
Gaur *et al.*[16]	1 (1)	61	5	Diarrhoea Weight loss Abdominal pain Fatigue	NT	0/NT	NT	NR	PVA IELs	NT	1	1/NR
Tran and Li[17]	1 (1)	NR	NR	Diarrhoea Weight loss	0	0/NT	NT	NR	TVA IELs	NT	NT	1/NR
Theophile *et al.*[18]	5 (3)	82 (78–87)	NR	Diarrhoea (4) Weight loss (4) Fatigue (2)	NT	NT/NT	NT	NR	PVA in 4 NV in 1 IELs in 2 (2PVA); NR in 3	NR in 3 pts; NA in 1* No improvement of symptoms in 1	NT	5/2 of 3 tested

Table 1 | (Continued)

Author (Reference)	*n* (M)	Age (yr) or mean age with range	Years of olmesartan intake or mean with range	Symptoms and laboratory findings (*n*)	HLA DQ2 or DQ8 positive (*n*)	TTG/EMA positive (*n*)	Anti-enterocyte antibodies positive (*n*)	Endoscopic findings (*n*)	Duodenal histology at diagnosis (*n*)	Amelioration of symptoms after after GFD (*n*)	Amelioration after steroid administration (*n*)	Clinical amelioration/ histological recovery after suspension of olmesartan (*n*)
Present study	3 (2)	66 (59–80)	3	Diarrhoea (3) Weight loss (2) Anaemia norm. (1) Anaemia micr. (1) Hypoalbuminaemia (2) Fatigue (3)	0	0/0	NT	Nodularity of mucosa: 2/3. TVA in 2 PVA in 1	TVA in 2 PVA in 1 IELs in 0	0	0 of 1 tested; NT in 2 cases	3/3

n, number of subjects; yr, years; TTG, transglutaminase antibodies; EMA, anti-endomysial antibodies; GFD, gluten-free diet; NT, not tested; NR, not reported; NA, not available; TVA, total villous atrophy; PVA, partial villous atrophy; IELs, more that 40% intra-epithelial lymphocytes; NSVA, not specified degree of villous atrophy; SCD, subepithelial collagen deposition; NV, normal villi.

* Concomitant discontinuation of olmesartan and start of GFD.

endoscopy after olmesartan suspension and in 25 (93%) of them a complete recovery of duodenal histology (including normalisation of both villous pattern and IELs infiltrates) was observed. None of the patients underwent olmesartan re-challenge to prove a causality relationship with sprue-like enteropathy.

DISCUSSION

Olmesartan intake has been recently associated with the development of a sprue-like enteropathy, mainly characterised by diarrhoea, weight loss and variable degrees of duodenal mucosa damage. In the present study, we performed a systematic review of the literature, including in the analysis three additional cases of olmesartan-associated enteropathy we diagnosed.

Our search found a total of 11 studies, all of which were case reports or series: this represents a limitation of our systematic review. Overall, 54 patients, equally distributed between men and women, have developed a sprue-like enteropathy associated with olmesartan treatment for arterial hypertension. Mean duration of olmesartan therapy was 3.3 years. Most common symptoms were diarrhoea and weight loss. Normocytic normochromic anaemia and hypoalbuminaemia were the most diffused laboratory alterations, being present in slightly more than a half of patients. Anti-endomysial and -transglutaminase antibodies were negative in 100% of patients, whereas the anti-enterocyte antibodies were positive, when tested, and with atypical pattern, in only three subjects. Similarly to the data shown by Rubio-Tapia,[8] HLA DQ2 or DQ8 haplotype was present in 72% of patients. Since in the normal population the DQ2/DQ8 prevalence is estimated at 30–40%,[20] our data suggest a possible role for genetics also in olmesartan-induced enteropathy.

Endoscopic pattern has been described by few authors only. We have already shown the effectiveness of both water-immersion technique[21] and i-scan technology[22] in the evaluation of duodenal villous pattern. Applying such tools, we found in two patients a nodular appearance of the duodenal mucosa (in addition to total villous atrophy), confirming data by Talbot *et al.* Since this finding has been reported in only three of 54 patients, it cannot be considered an endoscopic hallmark of sprue-like enteropathy. However, endoscopic features of the disease has been described just in four reports, and nobody except us made use of advanced endoscopic image techniques. Further studies should therefore include the application of endoscopic magnification tools for a better definition of such clinical entity.

Interestingly, variable degrees of villous atrophy were present in all patients but one (98%), whereas an increase in IELs infiltration into the duodenal mucosa was documented in only 65% of cases. In addition, a thickened subepithelial collagen band was found in the duodenum of one third (33%) of patients. If confirmed by subsequent, larger series, these findings may help to distinguish between coeliac disease and olmesartan-induced enteropathy from a histopathological perspective.

Such histological features may also suggest some conjectures about the pathogenic pathway of olmesartan-induced enteropathy, and its differences from coeliac disease.

Coeliac disease is an autoimmune disorder which is induced, in genetically pre-disposed people, by the ingestion of gluten which is rich in proline and glutamine. It

is characterised by an inflammatory reaction, primarily in the upper small intestine, with features of infiltration of the lamina propria and the epithelium with chronic inflammatory cells and progressive villous atrophy.[23] In the olmesartan-associated sprue-like enteropathy, the flattening of villi is instead not always associated with increase in IELs and inflammation.

The mechanisms underlying olmesartan-associated enteropathy are not known. At the present time, it is only possible to make some assumptions that have only a speculative value. Villous atrophy in the olmesartan enteropathy might be the result of a pro-apoptotic effect of angiotensin II on intestinal epithelial cells. Renin-angiotensin system is known to regulate fluid and electrolyte absorption in the human gut. Angiotensin II binds to two receptor forms, called AT1 and AT2, with different properties. AT1 receptor is expressed throughout the whole alimentary tract, while the AT2 receptor is expressed only in some tracts, particularly in the duodenum and jejunum.[24] Briefly, AT1 receptor activates growth-promoting factors and mediates major effects of angiotensin II, while AT2 receptor induces opposite effects.[25] Recently, Sun *et al.* have shown that angiotensin II promotes apoptosis of enterocytes through binding to AT2 receptor and consequent up-regulation of pro-apoptotic protein (Bax and GATA-6) associated with a down-regulation of Bcl-2, an anti-apoptotic protein.[26] In addition, drug-induced AT1 receptor blocking has been shown to exert translocation of AT2 receptors from cytosol to external membrane in presence of high concentrations of angiotensin II in rat smooth muscle cells; such behaviour may favours binding of angiotensin II to AT2 receptors.[27] Olmesartan shows high affinity for AT1 receptors. In case of AT1 receptor saturation by olmesartan, circulating angiotensin II could bind only AT2 receptor, with consequent pro-apoptotic effect. Apoptosis of enterocytes may ultimately lead to villous atrophy without inflammatory reaction and increase in IELs.

After the discontinuation of olmesartan, clinical remission occurred in 100% of patients, and almost all of them showed histological recovery of the duodenum (although if follow-up duodenal biopsy sampling has been performed in only a half of all cases). Steroid treatment induced improvement of symptoms in 19 patients, but none of them underwent subsequent endoscopic and histological assessment.

Olmesartan-associated sprue-like enteropathy may be considered as an adverse drug reaction. However, a cause–effect relationship should be classified only as probable according to the Naranjo probability scale, since neither olmesartan nor placebo have been reintroduced in none of the patients.[28]

In conclusion, olmesartan-associated sprue-like enteropathy may be considered as a distinct clinical entity, and should be included in the differential diagnosis of seronegative villous atrophy. Considering the worldwide use of olmesartan as anti-hypertensive treatment, olmesartan-associated sprue-like enteropathy may theoretically be widespread, and the clinical gastroenterologist should be aware of this. Up to now, however, only a few cases have been described, and scarcity of available data does not allow a correct definition of the disease. Areas of interest include the assessment of pathogenic pathways (including the role of the DQ2/DQ8 haplotypes), as well as of endoscopic and histological hallmarks of the disease.

AUTHORSHIP

Guarantor of the article: None.

Author contributions: Gianluca Ianiro, Stefano Bibbò and Giovanni Cammarota were involved in the study concept and design; in the analysis and interpretation of data; in drafting of the manuscript; in the critical revision of the manuscript, and in describing the clinical history of two reported cases. Massimo Montalto was involved in reporting the clinical history of a patient, and in the critical revision of the manuscript. Riccardo Ricci was involved in the histopathological analysis of the reported patients. Antonio Gasbarrini was involved in the critical revision of the manuscript. All authors approved the final version of the manuscript.

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Exhibit H

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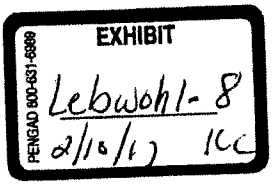
HYPERTENSION (S TALER, SECTION EDITOR)

Sprue-like Enteropathy Associated with Olmesartan



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Abstract Olmesartan-associated enteropathy (OAE) is a newly described condition reported in several case series in which patients taking olmesartan developed diarrhea, nausea, vomiting, dehydration, and weight loss. Although the symptoms and histologic findings of small-bowel enteropathy resembled severe celiac disease, laboratory work and the lack of response to a gluten-free diet challenged that diagnosis. The injury extended beyond the small bowel, with evidence of lymphocytic/collagenous gastritis and/or colitis in a substantial subset of patients. After a thorough diagnostic evaluation including consideration of alternate diagnoses, and resistance to a variety of treatments, a common thread became apparent: that all affected patients were taking olmesartan. Once this connection was recognized and the medication was suspended, symptoms would improve and the enteropathy healed. Some patients required corticosteroids particularly budesonide (a topically potent steroid) to achieve remission. There remains a gap in knowledge regarding the predisposing factors and mechanism of action.

Keywords Olmesartan · Diarrhea · Enteropathy

Introduction

Mechanisms of Diarrhea

Diarrhea is a commonly reported side effect of medications. Mechanisms depend on the type of medication, and for some medications, the mechanism remains undefined. In most circumstances, the diarrhea begins soon after the first use of the medication, and this temporal correlation lends itself to identification of the cause.

Osmotic Diarrhea

Medications such as magnesium-containing antacids and laxatives cause osmotic diarrhea, drawing water into the bowel lumen. Sorbitol, an artificial sweetener, can also induce osmotic diarrhea when used in excess. Often, these medications are chosen as treatment options for constipation based on their mechanism.

Secretory Diarrhea

Medications such as antidepressants, antiarrhythmic medications such as quinidine and digoxin, and antiasthmatic drugs increase active secretion in the small bowel.

Antibiotic-associated Diarrhea

Antibiotics, especially clindamycin and fluoroquinolones, are notorious for causing diarrhea by altering the gut flora, shifting the balance between protective, helpful bacteria and potentially harmful organisms. In its extreme form, *Clostridium difficile*, a bacteria naturally found in the large intestine, multiplies unopposed by the usual protective bacteria to dominate the new, altered gut flora. *C. difficile* releases toxins causing diarrhea and severe illness.

Malabsorptive Diarrhea

Patients taking azathioprine, mycophenolate mofetil, or methotrexate may develop villous atrophy of the small intestine [1, 2] with the resulting decreased small-bowel surface area and reduced absorptive capacity leading to malabsorption of

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protein, fat, and carbohydrates, even producing a secretory diarrhea if the crypts are enlarged. The inability of the mucosa to absorb nutrients leaves behind an osmotic load and induces diarrhea. Malabsorptive diarrhea is typically pale, bulky, and malodorous. Symptoms and histologic findings reverse with suspension of the immunosuppressant [1, 2]. By inhibiting lipase, the weight-loss medication orlistat prevents the absorption of fat, decreases the amount of calories absorbed, and leads to weight loss.

Olmesartan Mechanism and Kinetics

Olmesartan, a newer addition to the angiotensin II receptor blocker (ARB) class, entered the US market in 2002. It is administered as a prodrug, olmesartan medoxomil, and is converted to its active form, olmesartan, in the gastrointestinal mucosa during absorption. The active form undergoes no metabolism; bioavailability is low, and it is highly protein bound. Olmesartan has a 12,500-fold greater affinity for the AT1 receptor compared to the AT2 receptor and a greater relative affinity when compared to other earlier ARBs. The half-life is 13 h, also longer than other ARBs. It is sold under the trade name Benicar, or in combination with HCTZ as Benicar HCT, Azor when combined with amlodipine, and Tribenzor when combined with amlodipine and hydrochlorothiazide. Overseas, it is marketed as Olmetec. It is primarily used as an antihypertensive agent and is thought to have renal protective effects in diabetics. It may be used in combination with other agents. In several large clinical trials, olmesartan was found to have a favorable safety and side-effect profile. One study reported that olmesartan-associated adverse event rates and serious adverse event rates were similar to placebo with the exception of dizziness [3].

Clinical Presentation of Olmesartan-associated Enteropathy

The initial reports described severe diarrhea and profound weight loss as the cardinal presenting features of olmesartan-associated enteropathy (OAE) [4••, 5–9, 10•, 11–17, 18••, 19, 20•, 21••]. Patients had watery, non-bloody bowel movements occurring up to ten times daily persistent for weeks to years prior to diagnosis and suspension of olmesartan use. The typical patient had taken olmesartan for at least one and often many more years prior to onset of symptoms [4••]. Minimum and maximum duration until diagnosis was reported to range from 3 weeks to 53 months, respectively, with a mean duration of 19 months as reported in the initial case series [4••, 5]. Patients had significant weight loss, typically greater than 10 kg and up to 59 kg. Nausea, vomiting, and abdominal pain were common features. Some presentations were of abrupt onset and others more gradual, most often starting one or more years after the start of olmesartan therapy. Symptoms quickly progressed, causing escalating patient needs, some requiring hospitalization for their symptoms and complications of hypotension, acute kidney injury, vitamin and mineral deficiencies, and need for total parenteral nutrition (TPN). Hospital admission for hypotension prompted suspension of antihypertensive therapies, and thus alleviated patients' gastrointestinal symptoms which then recurred with the reintroduction of pre-admission medications including olmesartan. The key clinical features of OAE are displayed in Table 1.

A subset of patients had acute kidney injury, pre-renal in etiology, in the setting of hypokalemia [4••, 10•, 13]. The hypokalemia is likely secondary to the profuse diarrhea. Patients commonly had vitamin and mineral deficiencies and hypoalbuminemia [4••, 6, 8, 22] with 25 % having vitamin and mineral deficiencies including vitamin D, iron, zinc, magnesium, calcium, copper, vitamin B_{12}, vitamin A, vitamin C, and beta-carotene (Table 2). Symptoms and complications, especially malnutrition, often escalated, and with the inability to absorb micronutrients, some required parenteral nutrition.

Pertinent vital signs included hypotension and orthostatic hypotension. Physical exam findings included diffuse abdominal tenderness, further evidence of dehydration combined with lower extremity pitting edema due to hypoalbuminemia. Some patients were subjectively and objectively cachectic or underweight, but despite the significant weight loss as noted above, some patients remained overweight or obese. Otherwise, the physical examinations of these patients were largely unremarkable.

Diagnostic Evaluation

Patients underwent comprehensive evaluations, often at multiple institutions to determine the cause of their symptoms. Prior diagnoses included primarily celiac disease but also many other disorders (Table 3). Testing consisted of laboratory tests, imaging, and endoscopy with biopsies, typically showing an inflammatory enteropathy similar to celiac disease

Table 1 Clinical features of olmesartan-associated enteropathy (OAE)

Olmesartan use ≥1 year
Negative tissue transglutaminase (tTG)
HLA-DQ2+ status[a]
Diarrhea, nausea, and vomiting
Vitamin and mineral deficiencies
Small-bowel villous atrophy
Involvement of the stomach and/or colon
Recovery with olmesartan suspension and budesonide taper

[a] Although most patients described in the literature were HLA-DQ2+, there are patients that were HLA-DQ2-

Table 2 Nutritional deficiencies in patients with OAE seen at the Mayo Clinic

Hypokalemia	37.1 %
Vitamin D	34.3 %
Iron	31.4 %
Zinc	31.4 %
Magnesium	25.7 %
Calcium	14.3 %
Copper	14.3 %
Vitamin B12	8.6 %
Beta-carotene	8.6 %
Vitamin A	2.9 %
Vitamin C	2.9 %

(Fig. 1). Extensive, even exhaustive testing for alternative diagnoses was generally negative. Tissue transglutaminase (tTG) was typically negative [4••, 5–9, 10•, 11–17, 22]. Patients with OAE commonly carried the celiac-associated HLA-DQ2 or DQ8 [4••, 5, 6, 8, 12, 19] although not to the same degree as those with celiac disease. Abdominal CT scans were usually negative except in one case report in which a patient was noted to have colonic inflammation that led to perforation [22].

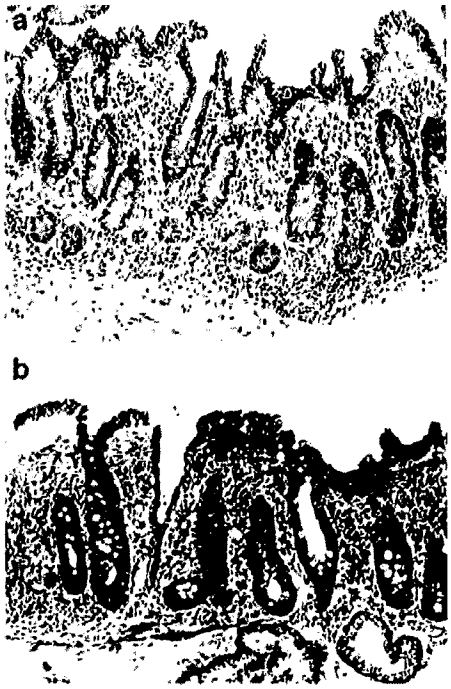

Fig. 1 The enteropathy associated with olmesartan is quite similar to that of celiac disease, illustrating severe villous atrophy, mucosal inflammation with crypt hyperplasia. Pictures courtesy of Tsung-Teh Wu, M.D., Ph.D., Department of Laboratory Medicine and Pathology, Mayo Clinic

Pathology

Endoscopic biopsies indicated a severe enteropathy often showing partial or total villous atrophy. One third of patients in the initial case series and two other reported cases had increased subepithelial collagen deposition in the small bowel [4••, 12, 19]. Indeed, the initial description of the association came from a series of patients with the extremely rare syndrome of collagenous sprue [23••]. Small-bowel aspirates detected small-bowel bacterial overgrowth [4••, 5], and about 30 % had gastric biopsy findings of unusual types of gastritis including lymphocytic, collagenous, eosinophilic, and mixed lymphocytic and collagenous gastritis.

One half of patients had small intestinal bacterial overgrowth although this could have resulted from breakdown of the mucosal barrier [4••, 5, 6]. Although the histologic findings were similar to celiac disease, one center noted that very few patients with OAE also had increased intraepithelial lymphocytes, seen in patients with celiac disease [24]. Lymphocytic or collagenous colitis were also common and often constituted the first diagnosis in these patients as colonoscopy was often the first test performed to evaluate the diarrhea [9, 10•, 22]. Duration of exposure to olmesartan was strongly correlated with the development of colitis (p=0.016).

Table 3 Alternate diagnoses patients were given and for which they received treatment prior to the diagnosis of OAE

Celiac disease
Lymphocytic or collagenous colitis
Gastritis
Small-bowel bacterial overgrowth
Collagenous sprue
Clostridium difficile
Autoimmune enteropathy
Refractory sprue
Tropical sprue
Crohn's disease

Etiologies/Risk Factors

One group found the risk of celiac disease diagnosis significantly increased after 1 year of exposure and thereafter in a large cohort of French patients with OAE who required hospitalization [18••]. The association of duration of exposure to symptom onset was an important finding, excluding other mechanisms of injury. OAE is not an allergic reaction to olmesartan as it does not have the typical features of an allergic response. OAE does not directly alter gut motility like macrolide antibiotics nor does olmesartan have a secretory effect that alters absorption.

This leads us to ask how olmesartan causes an abrupt onset of illness after a long duration of therapy. Certainly, the injury could be accumulating slowly over time with the patient only becoming symptomatic when the remaining amount of normal

bowel is no longer able to compensate for the damaged mucosa. Or olmesartan could have a role in gut immune homeostasis, predisposing the gut to a second hit that causes the abrupt presentation. This explanation may be more likely as it would support the sudden onset of symptoms of nausea, vomiting, and diarrhea patient experience, associated with small intestinal bowel overgrowth, and active inflammation that necessitated the use of steroids.

Celiac disease and other immune disorders are associated with certain HLA types. There appears to be a similar association with OAE and the same HLA type associated with celiac disease as most published cases were HLA-DQ2+ [4••, 5, 6]. This may be a spurious association due to selection bias but, if confirmed, suggests an immune basis for the disorder. Some patients had a positive ANA or other autoantibodies suggesting a perturbation in immune regulation that could uncover a predisposition to autoimmune disease as has been reported with an entirely different drug, ipilimumab, a TNF inhibitor [25].

Although the stomach and colon may also be involved, the site most severely affected is the small bowel, similar to celiac disease. While prolonged duration of drug exposure has been associated with colitis, other risk factors including dose, formulation, age, gender, and co-morbidities did not correlate with the development or severity of OAE. The rarity of the condition suggests either a genetic predisposition of the individuals or perhaps an uncommon coincidence of events that produce the syndrome.

Treatment

Given the diagnostic dilemma and obvious failure of a gluten-free diet, many medications were tried prior to the recognition of OAE. Prescribed medications included antibiotics, immunosuppressive agents, anticholinergics, opioid derivatives, probiotics, somatostatin analogs, bile acid sequestrants, antiemetics, and proton pump inhibitors. Forty percent of patients seen at the Mayo Clinic had taken an antibiotic at some point in the treatment of their symptoms. More than 20 % of patients reported use of diphenoxylate/atropine, pancreatic enzymes, and bile acid sequestrants. Although prescribed less often, immunosuppressive medications tried included 6-mercaptopurine, mesalamine, and azathioprine.

Once the possible role of olmesartan was recognized, the drug was stopped. Most, but not all, patients improved with drug withdrawal. Some more ill patients required budesonide (a topical potent corticosteroid) to initiate a clinical response, control diarrhea, and accelerate healing [4••, 11]. Most patients improved quickly, and some stopped taking the budesonide altogether due to the rapid improvement in their symptoms [4••, 5, 6]. Of our patients, 25 and 37 % reported symptomatic improvement in less than 1 week and by 3 weeks, respectively. This data is consistent with the improvement in symptoms described in other case reports.

Follow-up biopsies were taken at least 6 weeks, but usually 6 months after olmesartan suspension. Repeat endoscopic biopsies showed histologic improvement of the stomach, small bowel, and colon mucosa after olmesartan discontinuation [4••]. Table 4 shows the management of a patient with OAE.

Unfortunately, several patients had a prolonged course to symptom resolution or have yet to make a complete clinical recovery. These patients received infliximab infusions in addition to budesonide and required home parenteral nutrition.

After the diagnosis and treatment for OAE, several patients seen at the Mayo Clinic likely had underlying celiac disease as evidenced by symptoms with reinstitution of gluten into the diet and strong family history of celiac disease. One patient had a positive tTG, which normalized with continued adherence to a gluten-free diet. This subset of patients improved quickly while maintaining a gluten-free diet.

The initial case series noted anecdotal recurrence of symptoms with resumption of olmesartan use, and the FDA Mini-Sentinel report noted a positive re-challenge in 10 of 23 cases [4••, 26]. Patients were usually placed on an entirely new antihypertensive medication from a different class [4••].

Hypothesized Mechanism

The damage induced by olmesartan is a chronic inflammatory change similar but not identical to celiac disease. The small intestine is normally in a state of constant immune readiness to fight off all invading pathogens or noxious substances. Such a state if left unchecked would lead to marked tissue destruction if it were not heavily regulated. A key component of the normal regulation comes from the cytokine tissue growth factor beta (TGFb). TGFb is a predominantly regulatory cytokine that plays a crucial role in maintaining gut immune homeostasis through a variety of mechanisms and cell types [27, 28]. Since ARBs are known to inhibit TGF beta, olmesartan may disrupt gut immune homeostasis, favoring immune-mediated damage of the gastrointestinal tract. However, it cannot be solely caused by the inhibition of TGF beta

Table 4 Management of olmesartan-associated enteropathy (OAE)

1. Stop olmesartan
2. Monitor blood pressure and use non-ARB if needed for hypertension
3. Fluid repletion
4. Nutritional support
5. Add budesonide (the steroid needs to directly contact the affected mucosa, making the enteric-coated form of budesonide not effective as normally given)
6. Once better, stop gluten-free diet and taper budesonide over months

function as TGFb is also involved in driving collagen deposition in response to inflammation, something that is especially common in OAE. Further studies are needed to determine the role of TGF beta and other potential mechanisms of OAE. In understanding the mechanism of OAE, the multi-organ nature and, often, dramatic recovery need to be considered.

The search for a mechanism(s) needs to take into account that patients taking olmesartan also had reversible histologic changes to the stomach and colon. Interestingly, an animal study proposed that olmesartan may have anti-inflammatory and antioxidant effects in ulcerative colitis [29]. Higher doses of olmesartan produced greater effects, which were comparable or better than sulfasalazine. It is unknown if such a study would yield similar results in humans. Yet, our patients with OAE and without known inflammatory bowel disease developed colitis with exposure to olmesartan, and duration of exposure significantly correlated with having colitis. If similar findings hold true for people with ulcerative colitis, the different responses of the colonic mucosa to olmesartan may yield more information regarding predisposing factors and mechanisms of OAE. The usually quick recovery time of days to weeks also requires acknowledgement in determining the mechanism, as the insult would need to revert in a short duration yet allow for clinical and histological recovery.

Discussion

The recently recognized syndrome of OAE initially reported in case series and several case reports is indeed quite rare. The syndrome as reported is clinically severe and may be life threatening. It is likely immune based but not an allergic response to the drug. Once the association is recognized and the drug stopped, the disorder resolves. However, cases continue to occur, and despite numerous recent reports, the addition of an FDA warning in the labeling and several media stories, new cases still occur and remain unrecognized in clinical practice. Why does this happen? The great rarity of the disorder is one issue. Olmesartan and the other ARBs have been remarkably free of significant side effects and in general are well tolerated. Patients have often been on the drug for several years without incident so the lack of temporal correlation of diarrhea onset with the commencement of therapy makes it less likely that either the patient or their physician would recognize it. When intestinal biopsies are done and show enteropathy similar to celiac disease, treatment with a gluten-free diet is a slow treatment that even in the best of circumstances may have an uncertain response. In most cases, the presumptive diagnosis of either collagenous or refractory celiac disease serves as a ready although incorrect explanation for the lack of response. Indeed, this diagnosis could stick to the patient to the point of developing truly severe malnutrition requiring long-term parenteral nutrition. Clues to the diagnosis include the negative celiac disease serology (tTG). Serological tests need to be performed on a gluten-containing diet; if not done before the gluten-free diet is started, they would likely be negative. If positive HLA-DQ2 status is obtained, a more common finding with OAE, this further clouds the clinical picture as carriage of the genetic susceptibility type for celiac disease leads to even more certainty that the patient has celiac disease in a refractory state. The true nature of this syndrome evaded recognition in many specialized celiac disease centers including our own. It was likely the aggregation of many similar patients in a clinical practice largely devoted to refractory enteropathies, allied with the routine practice of medication reconciliation that led to the ultimate recognition of this hitherto unsuspected syndrome. Drug side-effect surveillance programs need to be searching for unexpected and rare events like this.

Once recognized, the patients' symptoms and histologic abnormalities quickly improved with suspension of olmesartan and a topically active budesonide taper. Their antihypertensive regimen was changed so as not to include an ARB.

Recognition of OAE as a clinical entity reminds us of several key lessons in caring for patients. Patients' symptoms were severe, unusual, and unexplained after extensive evaluation. In such circumstances, it is important to consider medication side effect in the differential diagnosis, regardless of how distant the time of initiation of medication to the onset of symptoms and how removed the constellation of symptoms may be from the primary pathway being targeted. Other medications such as fenfluramine-phentermine and bisphosphonates have been found to have serious side effects months to years after starting therapy and affecting organs distant from the site of target. In a 1997 New England Journal of Medicine, Connolly et al. published an association between women taking fenfluramine-phentermine for a mean of 12 months who developed cardiovascular symptoms or heart murmur secondary to changes in valvular morphology identified on echocardiography [30]. The affected valves resembled changes seen in patients with carcinoid syndrome or taking ergotamine. The bisphosphonates have been implicated in chemical esophagitis, and for some patients, the esophagitis was severe enough to require hospitalization [31]. Simple measures of staying upright for thirty minutes and taking each dose with a full glass of water reduce the risk of esophagitis. OAE, like the above historical examples, highlights the importance of considering medication side effects in patients with unusual symptoms and unrevealing diagnostic evaluations.

Patient accounts provide insight to providers and help determine the etiology of their symptoms. This was especially true in finding a connection between the patients' symptoms and olmesartan. Patients mentioned to their providers that their symptoms improved during hospitalization for severe

dehydration and hypotension, when their antihypertensive medications including olmesartan had been withheld, and worsened with restarting of olmesartan. Their anecdotes of waxing and waning symptoms in relation to olmesartan use aided in identifying its role in their presentation.

As mentioned earlier, many medications can cause diarrhea as a side effect. Patients with persistent diarrhea who are on a medication known to have diarrhea as a side effect warrant a trial off the medication while monitoring for improvement in symptoms.

These recent findings and severity of the symptoms raise the question of how to manage patients on olmesartan. First, patients on olmesartan without the cardinal features of OAE (i.e., diarrhea, weight loss, severe dehydration, and mineral and vitamin deficiencies) can likely continue olmesartan use. However, they should be informed of the presentation of OAE and the importance of notifying a health care professional if they develop these symptoms. Patients should also be regularly monitored for symptoms and signs of OAE, which can occur years after initial use. As clear risk factors are identified, recommendations may change. For patients with a diagnosis of celiac disease on olmesartan, the temporal relationship between olmesartan use and celiac disease diagnosis is important in determining the best course of action. Patients who were diagnosed with celiac disease prior to olmesartan use do not meet OAE criteria for explanation of their initial clinical presentation of celiac disease. Although the risk of developing OAE in the setting of celiac disease is not known, patients thought to have underlying celiac disease can be affected by OAE. If a patient with celiac disease on olmesartan develops symptoms suggestive of gluten exposure after an initial response to a gluten-free diet, they should be further evaluated for possible OAE. At that time, a repeat tTG, biopsy, and trial off olmesartan would be warranted in addition to identifying potential sources of gluten. On the other hand, if a patient was diagnosed with celiac disease while on olmesartan, they need re-evaluation to determine if their presentation could represent OAE rather than celiac disease, especially if they have negative celiac disease serology and are not responding appropriately to a gluten-free diet. In fact, one study suggested that medication-induced enteropathies accounted for 26 % of patients with villous atrophy in the setting of negative celiac disease serology, with olmesartan the identified culprit in 16 of 19 patients [32]. Patients presenting with diagnostic criteria of OAE (Fig. 2) require further evaluation with history, physical exam with particular attention to signs of dehydration and malnutrition, laboratory work that supports a diagnosis of OAE (i.e., tTG) or screens for potential complications of OAE (i.e., electrolyte panel and creatinine), and a trial off olmesartan. Figure 2 shows an approach to care for the patient taking olmesartan. Mini-sentinel, the post-marketing surveillance program of the Food and Drug Administration (FDA), acknowledged an increase in reporting of new cases of celiac disease in patients taking olmesartan in 2013. Data for exposure time and celiac disease diagnosis in patients taking olmesartan, candesartan, eprosartan, irbesartan, losartan, telmisartan, valsartan, hydrochlorothiazide, and atenolol are being collected. In November 2012, a Mini-Sentinel rapid data report did not show a significant increased risk of celiac disease in patients taking olmesartan compared to other ARBs. However, a follow-up study did show a significant risk [26]. This is consistent with a French nationwide study of 4.5 million cases over 5 years that concluded that the risk of developing enteropathy correlated with olmesartan treatment duration, with significant risk after 1 year [18••]. No other ARBs were implicated in the study [18••]. On July 3, 2013, the FDA approved new labeling for olmesartan that includes a warning of sprue-like enteropathy. Diarrhea is a recognized class side effect of the ARBs, but the sprue-like enteropathy warning does not appear on the label of other ARBs.

Further Work to Be Done

The clinical presentation and histologic findings of OAE are well described in a case series and several case reports. Information regarding predisposition, the potential of other ARBs, and potential mechanism are still lacking, and identifying key concepts in one area may provide insight to the others.

Predisposing factors needing further study include HLA-DQ2 status, the multi-organ nature and severity of disease, and for some the decreased response to olmesartan withdrawal and budesonide taper. Duration of exposure is the only known risk factor, independent or dependent, in developing OAE-associated colitis [18••]. It may also have a dependent role in the occurrence of OAE in general as most patients had at least a 1-year exposure. Although HLA-DQ2+ status has not correlated with the development or severity of OAE, more patients than expected carry a positive genotype. This could represent a referral bias, but additional studies are needed to determine its role in predisposition. No known factors predict the involvement of stomach or colon (with the exception of duration of use), vitamin and mineral deficiencies, acute renal failure, need for parenteral nutrition, hospitalization, and decreased response to olmesartan withdrawal and budesonide taper.

The current literature does not report a similar entity in response to other ARBs. However, recent anecdotal experiences of the senior author suggest that other ARBs—irbesartan, valsartan, and telmisartan—could be implicated. Some patients taking these medications have had a similar clinical and histologic presentation as OAE. Providers recognized the role of the medications in the patients' symptoms, and symptoms resolved with ARB suspension. Unfortunately, one patient taking olmesartan underwent numerous diagnostic tests and treatments without identifying an etiology for the

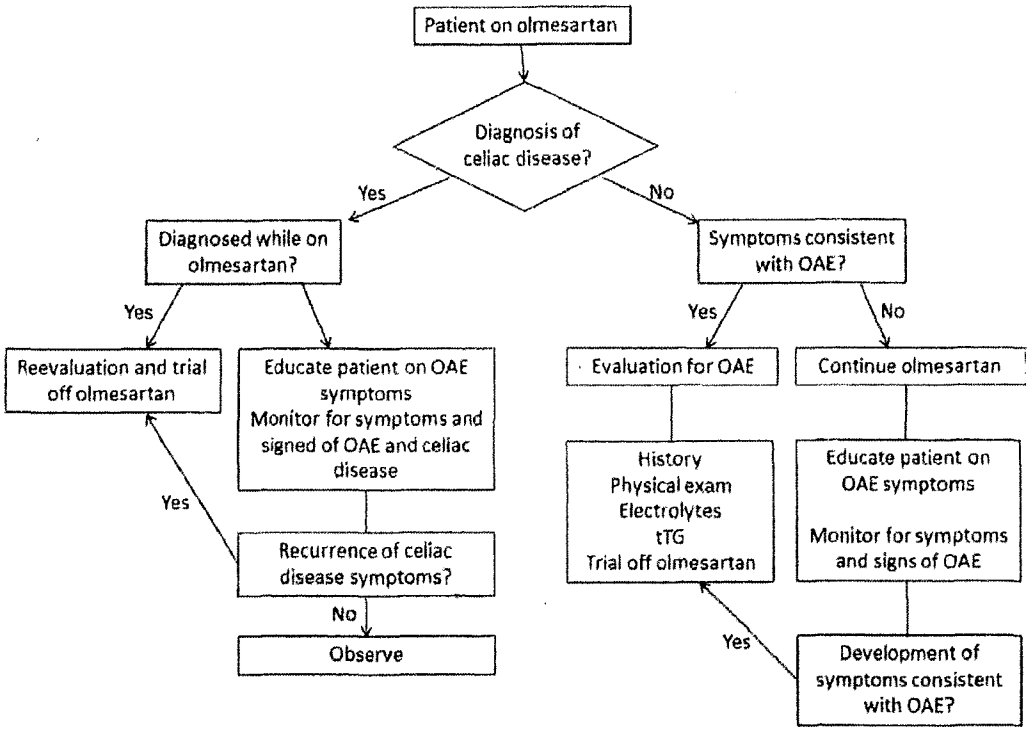

Fig. 2 Approach to the patient taking olmesartan. An algorithm to monitor patients for the development of OAE. Note the clinical features of olmesartan-associated enteropathy (OAE) in Table 1 and further description of symptoms in the section "Clinical Presentation of Olmesartan-associated Enteropathy". Figure 2 does not flow well on the left section. After the box "Educate the patient on OAE...," it is unclear why there would be a "No" connection to the recurrence of celiac symptoms. You would need two boxes saying recurrence of symptoms or no symptoms and then connect to a trial off olmesartan. You could use the same approach as on the right and remove the "No" comment

enteropathy. During one hospitalization, telmisartan was used instead of olmesartan, which was not on the hospital formulary. His symptoms persisted while taking telmisartan. Despite immunosuppressive therapy and TPN, the patient died. Autopsy reports and cause of death were not available for review. Increased awareness that other ARBs may induce similar symptoms is necessary to identify such cases. Recognition of the role of other ARBs may alter how one approaches searching for the mechanism of injury. Is this a class effect of ARBs or specific to olmesartan?

These aspects deserve consideration in determining the mechanism or mechanisms of OAE. Specifically, the mechanism needs to account for mucosal damage in three different organs. The stomach, small bowel, and colon have different cell types and cellular environments to accommodate their varied functions. Injury may occur by different means depending on the location, and if so, an assessment for predisposing factors should ensue. An ideal mechanism would reflect the patients' acuity of symptoms and the often-dramatic recovery. Lastly, exposure to other ARBs may lead to similar symptoms, and if so, the mechanism of OAE needs to reflect this commonality rather than rely on distinct chemical properties unique to olmesartan. However, the intestinal activation of olmesartan, its relatively high potency, and the prodrug formulation with medoximil which until recently was unique among ARBs may explain the association of the syndrome almost exclusively with olmesartan.

Conclusion

Medication side effects commonly include diarrhea, and the mechanism is often unknown. Prescribers and users of olmesartan need to be aware of the potential for a rare but potentially severe side effect of this diarrhea illness and promptly seek care and evaluation when it occurs. It would seem reasonable to hold olmesartan much earlier in the natural history of the illness rather than assuming other diagnosis first in order to limit worsened symptoms leading to nutritional deficiencies and requiring a greater level of care and more extensive diagnostic evaluations. Patients' symptoms tend to resolve quickly, often within 1 month after the suspension of olmesartan. In those with a more severe disease, a budesonide taper seems to accelerate recovery. A small subset of patients does not recover spontaneously. Thus, it behooves us as clinicians to consider medication effects no matter how

unrelated they seem, when a patient develops an unusual syndrome even if outside of our discipline

Compliance with Ethics Guidelines

Conflict of Interest Joseph Murray and Amanda Cartee have no disclosures relevant to this work.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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Exhibit I

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November 30, 2016

Via Electronic Service

Susan Sharko, Esq.
Drinker Biddle & Reath LLP
600 Campus Drive
Florham Park, New Jersey 07932-1047

Re: **In re Benicar (Olmesartan) Products Liability Litigation,**
MDL No. 2606
NJ MCL Case No. 299

Dear Ms. Sharko:

Please find attached the Expert Report of Daniel Leffler, M.D., served on behalf of Plaintiffs in the above-referenced litigations.

Sincerely,

Tara Sutton

Tara D. Sutton

cc: Jessica Brennan, Esq. (*via electronic mail*)
Michael Zogby, Esq. (*via electronic mail*)
Adam Slater, Esq. (*via electronic mail*)
Chris Coffin, Esq. (*via electronic mail*)
Rayna Kessler, Esq. (*via electronic mail*)

Attachment

EXPERT REPORT OF DANIEL LEFFLER, M.D.

TABLE OF CONTENTS

I. EDUCATION AND EXPERIENCE

1. I am the Director of Research at the Celiac Center and the Director of Quality Improvement for the Division of Gastroenterology at Beth Israel Deaconess Medical Center, in Boston, Massachusetts. I am an Associate Professor of Medicine at Harvard Medical School and serve as the Associate Director of Research for Quality Improvement, Department of Medicine. I am the Associate Firm Chief in the Department of Medicine at Beth Israel Deaconess Medical Center. I am certified in Gastroenterology by the American Board of Internal Medicine since 2008 and serve on the Intestinal Disorders Section for the American Gastroenterological Association.

2. In July, 2016 I accepted a position as a Medical Director in the Gastrointestinal Therapeutics Area at Takeda Pharmaceuticals. In this role, I assist in the evaluation of the clinical efficacy and safety of novel therapies for gastrointestinal diseases.

3. Over the past decade I have worked extensively in intestinal diseases, focusing on celiac disease and related intestinal malabsorptive disorders. I have treated hundreds of patients with chronic digestive disorders and routinely perform endoscopic procedures for diagnosis and management. I have served as a clinical investigator in studies involving a number of issues, including: gastrointestinal endoscopy safety; gluten free diet adherence; HLA testing in celiac disease; mesalamine as a treatment for celiac disease; celiac disease biomarkers; and celiac disease diagnosis education. I am a founding member and past Secretary for the North American Society for the Study of Celiac Disease, on the medical advisory board for the National Foundation for Celiac Awareness and the Celiac Disease Foundation and served on the Steering Committee Member for the FDA Gastrointestinal Regulatory Endpoints and Advancement of Therapeutics Conference on Celiac Disease.

4. I have authored and edited more than 100 scientific publications on gastroenterology topics, predominantly on celiac disease and related small intestinal disorders including clinical presentation, diagnosis, management and pathophysiology. I have also authored multiple textbook chapters including one on celiac disease for the 2015 Yamada's Textbook of Gastroenterology, 6th ed, and one on small intestinal diseases including celiac disease for the Digestive Diseases Self-Education Program (DDSEP) 8 for the American Gastroenterology Assocation. I am currently on the editorial board for Clinical Gastroenterology and Hepatology and the World Journal of Gastroenterology and serve as an ad hoc reviewer for more than 20 other peer reviewed journals including the New England Journal of Medicine, the Journal of the American Medical Association, and Gastroenterology. My *curriculum vitae* is attached.

5. I have received numerous honors and awards in my career. I have, for example, received: the Presidential Poster Award from the American College of Gastroenterology (2006); AstraZeneca Senior Fellow Abstract Award, American College of Gastroenterology(2007); PCIR Junior Investigator Laboratory Support Award, Harvard Catalyst (2010); Irving W. and Charlotte F. Rabb Prize for Gastroenterology Research (2012); Dvorak Young Investigator Award for Health Services Research (2013); Journal Author Excellence Award, American Society for Healthcare Risk Management (2013); and the Nezam Afdhal Outstanding Achievement for Innovation in Gastroenterology Award (2015).

6. I received a Master's Degree in Nutrition at Columbia University in 1998 followed by an MD from the combined program though Columbia University and Ben Gurion University. I subsequently completed my internship and residency in Internal Medicine in 2005 and my fellowship in gastroenterology in 2008, all at Beth Israel Deaconess Medical

Center in Boston. I was supported by an NIH clinical investigator career development award from 2009-2014.

7. I have been asked to review the scientific literature and certain adverse event information in the possession of the defendants in these cases, to determine whether olmesartan can cause inflammation and damage to the small intestine resulting in malabsorption, diarrhea, abdominal pain, weight loss, vomiting and related symptoms. Relevant to this, I have cared for patients with severe malabsorption due to olmesartan induced inflammation in the lining of the small intestine, referred to below as "olmesartan enteropathy" but also previously known as "sprue-like enteropathy." "Enteropathy" is the preferred medical terminology for pathologic changes in the lining (mucosa) of the small intestine. The term "sprue" was historically used to denote poorly understood intestinal disorders, but lacks a concrete medical definition. As the link between olmesartan and enteropathy has become accepted, "olmesartan enteropathy" has largely replaced "sprue-like enteropathy" in the medical literature. Olmesartan enteropathy, in my practice, was often misdiagnosed as one or a combination of celiac disease, refractory celiac disease, inflammatory bowel disease, collagenous colitis, microscopic colitis, lymphocytic colitis, autoimmune enteropathy or irritable bowel syndrome. The severity of symptoms I have observed in clinical practice range from chronic diarrhea and abdominal pain without weight loss, to severe malabsorption requiring multiple hospitalizations, and in one case cardiac arrest due to dehydration and electrolyte disturbances from severe diarrhea. All of the patients I have treated have had resolution of clinical malabsorption after cessation of olmesartan. In particular, looking back at a publication from my research group on refractory celiac disease, two patients were on olmesartan and likely had olmesartan enteropathy rather

than true refractory celiac disease and one of these patients eventually had olmesartan stopped, after which her gastrointestinal disease resolved, the second patient was lost to follow up.

8. I express each opinion to a reasonable degree of medical certainty. In making these determinations, I have followed the same procedures that I employ in my clinical practice. I have also used the same procedures that I employ in the clinical study of celiac disease and related gastrointestinal diseases.

II. RELATIONSHIP BETWEEN SMALL INTESTINAL INFLAMMATION, GASTROINTESTINAL SYMPTOMS AND MALABSORPTION

9. The normal small intestine is lined with tiny fingerlike projections called "villi." Villi dramatically increase the absorptive surface area of the small intestine and produce many enzymes necessary for digestion. Between the villi are pit-like "crypts" which are responsible for regenerating damaged villi. In the normal intestine the villi are approximately 4 times the length of the crypts. As the intestine is damaged, the villi become shorter and the crypts become deeper as they hypertrophy to maintain sufficient villous structure. The healthy intestinal lining also contains all types of inflammatory cells at low numbers. The most prevalent inflammatory cell in the small intestine is the lymphocyte, often referred to as the "intra-epithelial lymphocyte" or IEL. A picture of normal intestinal lining can be seen in Figure 1.

10. Inflammation of the small intestine can be caused by immune mediated diseases such as celiac disease or Crohn's disease, allergic/eosinophilic conditions, infections such as giardia or norovirus, immune deficiency syndromes such as Common Variable Immune Deficiency, and drugs including olmesartan. Histologic characteristics of small intestinal inflammation, also known as enteropathy, can include one or more of the following features: 1) increase in intraepithelial lymphocytes, 2) architectural disruption of the lining of the small

intestine with shortening, blunting or atrophy of villi, 3) lengthening of crypts between villi, 4) increase in subepithelial collagen deposition, 5) neutrophil infiltration with or without crypt abscesses or granulomas, 6) loss of goblet and paneth cells, and 7) increased eosinophils. These changes are not mutually exclusive; patients will often have more than one of these findings, and changes may be similar across different diseases. Changes are sometimes patchy in nature with areas of severely affected intestine in close proximity to areas which are normal or near normal. This patchy nature of inflammation occurs across many immune mediated disorders both inside and outside of the gastrointestinal tract.[1] A picture of damaged intestinal lining can be seen in Figure 2.

11. The diagnosis of olmesartan enteropathy is currently made clinically based on the presence of typical gastrointestinal symptoms and signs of malabsorption in patients taking olmesartan. Biopsy of the small intestine is not necessary if the presentation is typical for olmesartan enteropathy and the patient responds to olmesartan cessation. However, biopsy can be helpful in ruling out other disorders or to confirm response to withdrawal of olmesartan. While biopsies of the small intestine may diagnose enteropathy, biopsies are able to evaluate less than one $10{,}000^{th}$ of the lining of the small intestine and we are unable to quantify the overall burden of damage over the entire intestine. This is one reason why the degree of enteropathy on biopsy is often not well correlated with severity of signs and symptoms of disease, and biopsy abnormalities can be minimal or non-existent, even in cases of clinically apparent olmesartan enteropathy, due to patchiness of disease.[2,3] Lack of awareness by doctors about olmesartan enteropathy may delay evaluation and diagnosis, as may the presence of only mild to moderate symptoms. Some studies suggest that patients without severe symptoms or

severe enteropathy on biopsy are still at risk for complications of malabsorption, including anemia, osteoporosis and complications of chronic inflammation including lymphoma.[4,5,6,7,8]

12. As the relationship between olmesartan and gastrointestinal symptoms and malabsorption has become more established, my current medical treatment is discontinuation of olmesartan and clinical observation. If patients do not respond to withdrawal of olmesartan, then further evaluation including endoscopy should be considered. This approach was recently set forth in the international 'Bucharest Consensus on Microscopic Enteritis'.[4]

13. Some adults with olmesartan enteropathy will be symptomatic without malabsorption. These individuals may have a range of symptoms including bloating, abdominal pain, vomiting, diarrhea, nausea, constipation, fecal incontinence and fatigue. These symptoms may wax and wane or be progressive over time, and are often attributed to irritable bowel syndrome before olmesartan enteropathy is diagnosed.

14. Other patients with olmesartan enteropathy will progress to malabsorption, defined as lack of proper absorption of food in the small intestine. Malabsorption can be caused by deficiencies in digestion, as with pancreatic exocrine insufficiency, where there is a lack of necessary digestive enzymes, or by decreased absorption due to olmesartan enteropathy. Symptoms of malabsorption can be acute or chronic. Acute symptoms include diarrhea, steatorrhea (fat malabsorption), weight loss, neuropathy and weakness/fatigue, and acute signs and laboratory manifestations include weight loss, anemia, rashes, dry skin, hair loss, kidney or liver injury and osteoporosis. While some of these issues, such as diarrhea, generally resolve with cessation of olmesartan, adverse health effects of moderate to severe malnutrition may persist. Chronic health effects which may persist even after normalization of nutritional status include neuropathy, weakness and fatigue, kidney injury, liver injury from

TPN (intravenous nutritional support), irritable bowel syndrome, fecal incontinence, neuropathy and osteoporosis. Each of these health outcomes may be caused by one or more issue related to olmesartan enteropathy. For instance, diarrhea in these cases is caused by a combination of the osmotic effects of unabsorbed food as well as active secretion related to inflammation. Anemia may be related to one or more vitamin deficiencies including iron, B12 and folate as well as direct effects of chronic inflammation. Renal injury is related to low blood pressure as well as electrolyte deficiencies. Acute liver injury may be caused by malnutrition itself, while chronic liver injury is a common consequence of TPN. Neuropathy, hair and skin changes are generally directly related to nutritional deficiencies such as B12 and zinc. Osteoporosis can be related to systemic inflammation as well as vitamin D deficiency, and often will not improve in older adults once bone mass is lost.

15. Even after clinical malabsorption and malabsorptive symptoms including diarrhea resolve after cessation of olmesartan, gastrointestinal symptoms including chronic abdominal pain and altered bowel habits consistent with irritable bowel syndrome may persist for years, as is seen after acute gastrointestinal infections.[9] This post-inflammatory irritable bowel syndrome is differentiated from enteropathy by the lack of malabsorption and, if done, normal intestinal biopsies. It is notable that all the symptoms and medical issues discussed above are directly related to the enteropathy and malabsorption.

16. In addition, there may be multiple severe complications of therapy for malnutrition. As mentioned above, TPN can lead to permanent liver damage, and can also lead to severe, life threatening infections. Hospitalization and the need for intravenous access for fluids, electrolytes and nutrition can increase the risk of blood stream infections and blood

clots. Medical treatment and the illness itself results in significant economic and social consequences with missed work, loss of livelihood and social isolation.

17. These issues are compounded by frequent misdiagnosis of celiac disease in patients with olmesartan enteropathy and prescription of a gluten free diet. The gluten free diet has been reported to be nutritionally deficient in several studies.[10,11,12] Specific nutritional issues commonly found in the gluten free diet include weight gain related to high-calorie gluten free substitute foods, elevated homocystine related to lack of b vitamins, and low calcium and iron.[10,13,14,15]

18. Quality of life is also significantly compromised, both by chronic gastrointestinal symptoms and, where celiac disease is misdiagnosed, by the burden of constant dietary restriction. A number of studies have reported a diminished quality of life associated with both celiac disease and the gluten free diet.[16,17,18,19] Data overall confirm that diagnosis of celiac disease has a significant psychological impact and that anxiety and depression may be ongoing issues in patients with celiac disease, affecting treatment adherence and overall quality of life.[19]

19. In addition to the specific quality of life impact of celiac disease diagnosis, there are also clearly documented effects of chronic gastrointestinal symptoms on quality of life and social function.[20] The burden of chronic gastrointestinal symptoms can be quite severe, with quality of life in affected patients similar to those with end stage renal disease or diabetes mellitus.[21,22]

20. Beyond these acute consequences of olmesartan enteropathy, there are also significant long term sequelae of malnutrition which can affect nearly every organ system. Complications commonly seen in malnutrition include:

Cardiovascular	Hypotension, mitral valve prolapse, arrhythmias, heart failure
Dermatologic	Dry skin, alopecia, lanugo hair, pruritus
Gastrointestinal	Constipation, delayed gastric emptying, hepatitis, dysphagia
Endocrine and metabolic	Amenorrhea, infertility, osteoporosis, thyroid and cortisol abnormalities, hypoglycemia
Hematologic	Pancytopenia (anemia, immune suppression, increased bruising and bleeding)
Neurologic	Cerebral atrophy
Pulmonary	Aspiration pneumonia, emphysema related to vomiting
Psychological	Depression, irritability, apathy

Table 1.[22,23]

A few of the more common and clinically significant effects of malnutrition include bone disease and immune suppression. Malnutrition causes impaired immune response though a variety of mechanisms including decreased number and function of immune cells leading to infection.[24,25] Poor nutrition also can impair gut barrier function and increase intestinal permeability, which can lead to infection, liver damage and constitutional symptoms.[25, 26,27]

III. IMMUNOLOGY AND PATHOGENESIS OF OLMESARTAN ENTEROPATHY

21. Enteropathy results from an inflammatory process with several etiological triggers. Recruitment and activation of intra-epithelial lymphocytes is pivotal and it is likely there are etiology-specific factors with a unique pro-inflammatory cytokine profile and triggering antigens. Enteropathy in celiac disease appears to be the closest match for the changes seen with olmesartan and is the best-understood related disorder. Celiac disease and olmesartan enteropathy are similar in that both involve the following: 1) stimulation of antigen-presenting cells to mature and express increased co-stimulatory molecules; and 2) a key cytokine in both celiac disease and olmesartan enteropathy is interleukin-15.

22. IL-15 is a central mediator in the innate to adaptive immune pathway. IL-15 is present in the epithelium and submucosa of the intestine and acts on multiple cell types

though multiple mechanisms to cause inflammation.[28,29] In the normal immune system TGF beta is an important regulator of immune surveillance and prevents autoimmunity though suppression of T helper cells and promotion of regulatory T cells. However, elevated levels of IL-15, as described in olmesartan enteropathy[30] as well as celiac disease, leads to loss of immune homeostasis.[28,31,32] This can occur even in the presence of normal TGF beta levels, either though TGF beta independent mechanisms, or though disruption of TGF beta signaling.[29,32]

23. Substantive differences between celiac disease and olmesartan enteropathy include the following: 1) in celiac disease, environmental triggers such as viruses, bacteria and possibly gluten itself activate antigen-presenting cells including dendritic cells and epithelial cells. In olmesartan enteropathy, permissive environmental triggers may in some cases trigger the onset of enteropathy which is not dependent on gluten. 2) In celiac disease, Transglutaminase 2 and gluten peptides form complexes, which B cells internalize and then present the complexes on the HLA-DQ2 or HLA-DQ8 molecules on their surface. These gluten-specific B cells can bind with gluten-specific CD4+ T cells and stimulate differentiation of B cells into plasma cells, which produce antibodies both to gluten peptides and to the self-protein Transglutaminase 2. In olmesartan enteropathy, there does not appear to be cross reactivity with Transglutaminase 2, and no subsequent production of anti-transglutaminase antibodies, as in celiac disease. Additionally, while there does appear to be an association between HLA DQ2/DQ8 and olmesartan enteropathy, this is not nearly as strong as in celiac disease. This suggests that, unlike celiac disease, olmesartan enteropathy is either not HLA-dependent or is able to bind to a wider range of HLA molecules.

24. To summarize, olmesartan causes an enteropathy which is pathologically highly similar to celiac disease and appears to share key mechanisms triggering an interleukin 15-mediated inflammatory response. At the same time, there are notable differences between celiac disease and olmesartan enteropathy, including dependence on Transglutaminase 2 and HLA DQ2/DQ8 in celiac disease but not olmesartan enteropathy. In clinical practice, olmesartan enteropathy rather than celiac disease is suggested by response to withdrawal of olmesartan and lack of response to a gluten free diet and may be supported by the absence of celiac disease serologies such as tTG and DGP, and testing negative for HLA DQ2/DQ8. Family and personal medical history can also be supportive as many patients with celiac disease have a personal and/or family history of celiac disease or other autoimmunity, where this is uncommon in patients with olmesartan enteropathy.

25. In my clinical work and research studies, I often assess the relationship between exposures (to foods, medications and infections), gastrointestinal symptoms, and endoscopic/pathologic findings. In determining a potential causal relationship, I frequently take into account the following factors[33]:

- Is there a plausible biological mechanism for this adverse effect;
- Is there evidence of withdrawal and challenge effects such that the symptoms/enteropathy improve with removal of the suspected agent and relapse with repeat exposure;
- Is there a temporal or dose response relationship between the exposure and the symptoms/disorder;
- Is there a known relationship between the exposure and the symptoms/disorder, either in medical literature or on the drug label;
- Are there other likely alternative explanations for the adverse effect.

IV. OLMESARTAN CAUSES ENTEROPATHY

26. It is my opinion, held to a reasonable degree of medical certainty, that olmesartan causes enteropathy with related gastrointestinal symptoms and malabsorption. I base my opinion on the following evidence:

A. Plausible Biologic Mechanism

27. While studies into the pathogenesis of olmesartan enteropathy are ongoing, data suggest that the mechanism is similar to that of celiac disease.[30,34,35] In olmesartan enteropathy, as with celiac disease, it is clear that there is a profound increase in cytotoxic CD8+ T cells, which together with granzyme B+ cells are the main mediators of damage to the intestinal epithelium. Olmesartan also appears to increase expression of IL15 and IL15R, which are key regulators of intestinal immune function.[30] Indeed, IL15 has been considered to be a 'master regulatory cytokine', and overexpression of IL15 promotes the initiation and perpetuation of destructive T cell responses as described above.[36] Additionally, it was shown that olmesartan, but not related medications telmisartan and losartan, increased production of interleukin 15 with subsequent increased numbers of local CD8+ T cells, and resulted in disruption of the tight junctions between enterocytes lining the intestine, consistent with this mechanism.[30]

28. In addition to the documented direct effects of olmesartan on key lymphocytes and cytokines, across multiple case series, 68-92% of patients diagnosed with olmesartan enteropathy have been found to be HLA DQ2 or HLA DQ8 positive, compared to 30-40% of the general population and 99% of patients with celiac disease.[3,37,38] While the HLA linkage is not as strong as with celiac disease, this, in combination with the fact that celiac disease is histologically highly similar to olmesartan enteropathy, suggests that there may be olmesartan-related compounds which bind with high affinity to certain HLA molecules and further

promote the inflammatory reaction described above. In this model, olmesartan has the innate ability to upregulate mediators of intestinal inflammation with variable penetrance facilitated by, but not reliant on, environmental, HLA and likely other genetic factors. This immune activation leads to the increase in intraepithelial lymphocytes and villous destruction seen in olmesartan enteropathy, and is generally distinct from the findings in allergic/eosinophilic disorders or Crohn's disease.

B. Evidence of Temporal Response, Withdrawal and Rechallenge Effects

29. As is expected with autoimmune conditions, there is significant variation in time to onset of olmesartan enteropathy, and overall risk increases with duration of use. As reported in a study of the French National Health Insurance claim database evaluating all adult patients initiating ARB or ACEI between 1 January 2007 and 31 December 2012, the risk of olmesartan enteropathy increases with duration of exposure to olmesartan.[39] This is the pattern expected of intestinal adverse reactions from medications. Either a cumulative dose effect which triggers olmesartan enteropathy or a second environmental trigger could explain these epidemiological findings. This need for a 'second hit,' where an individual is predisposed to an inflammatory condition but does not manifest it until the immune system is primed by a secondary stimuli such as infection or stress, is common in allergy and autoimmunity.[40,41] The lack of differential risk of olmesartan enteropathy by daily dose suggests that all clinical regimens are above the threshold for reactivity. This is similar to celiac disease where the threshold for reaction to gluten is well below common daily intake such that the amount and timing and gluten exposure is not a clear risk factor for individual development of celiac disease.[41]

30. As confirmed by multiple published case reports and in my clinical practice, olmesartan enteropathy is characterized by positive dechallenge and rechallenge effects, a

pattern of great clinical significance for causation. Once olmesartan enteropathy is identified and olmesartan is withdrawn, there is gradual improvement in both symptoms and intestinal inflammation. The rate of improvement can vary between individuals such that some patients are markedly better within days of stopping olmesartan and others take weeks or even months to substantially improve. This variability in response to withdrawal is commonly seen in other gastrointestinal disorders including celiac disease and may, in part, relate to degree of nutritional compromise at diagnosis. Finally, when rechallenge is attempted, most reports note very quick recurrence of gastrointestinal symptoms, usually within hours or days of exposure. This is also highly consistent with celiac disease and is related to an immune system that is already primed to react against an antigen.[42] The time course for the onset of olmesartan enteropathy, as well as the response to both withdrawal and rechallenge, are very consistent with what would be expected for an antigen-based immune mediated enteropathy.

V. ADVERSE EVENTS REPORTED TO DEFENDANTS PROVIDE EVIDENCE OF CAUSATION

31. As part of my review, I assessed Medwatch forms relating to gastrointestinal adverse events experienced by patients taking olmesartan. Overall, these reports are highly consistent with the cases reported in peer reviewed literature, with the exception that these reports were submitted as early as 2004, while the first clear description of olmesartan enteropathy appeared in the medical literature 8 years later in 2012.[38] It is also notable that many early cases as early as 2004 have medically severe outcomes including hospitalization and very convincing evidence of early recurrence of symptoms with rechallenge. My review of these MedWatch reports supports my conclusion that olmesartan causes enteropathy. In addition, I was asked by Dr. David A. Kessler on 11/01/2016, to review 62 Medwatch cases of potential olmesartan enteropathy, selected because they had at least one of the symptoms of diarrhea,

vomiting or celiac disease either in the coded preferred terms or in the narrative discussion, positive rechallenge data and a serious outcome. These criteria are ones I consider highly relevant to diagnosis of olmesartan enteropathy. I reviewed each of these reports, and based on the information available, I performed a differential diagnosis on each to rule out other plausible, alternative causes, and we discussed my review by telephone on 11/22/2016. In this discussion, I confirmed that 60 of these 62 identified cases were highly consistent with olmesartan enteropathy, both by clinical syndrome and response to olmesartan withdrawal and rechallenge. One of the cases was excluded because the clinical syndrome of constipation, intestinal obstruction and pancreatitis was inconsistent with olmesartan enteropathy. The second case was excluded because the very fast onset of symptoms, within one week of starting olmesartan, is unusual with olmesartan enteropathy. Below, I have summarized five cases which are representative of the collection as a whole.

a) A 58 year old woman had been treated with Benicar HCT 40/25 from the winter of 2003. In April 2004 she developed severe diarrhea. She was diagnosed with giardia infection, however a course of metronidazole did not improve her symptoms and she was hospitalized for dehydration in late April 2004. She stopped the Benicar at that time and symptoms resolved. After initial cessation the patient reports taking Benicar 'once in a while' for increased blood pressure. On each occasion she became "violently ill with vomiting and diarrhea." Since the patient and her physician concluded that these symptoms were due to the Benicar, no further episodes were reported. (Case SU-2004-002638.)

b) A 56 year old woman had been treated with olmesartan medoxomil as part of a clinical trial SE-866/44 (ROADMAP) starting on January 13, 2006. On November 1, 2006 the patient developed 'gastroenteritis' and hypokalemia. Olmesartan was discontinued on November 6, 2006 and the patient was hospitalized from November 17, 2006 to November 28, 2006 and symptoms were reported to have resolved by December 1, 2006. Gastrointestinal symptoms recurred when olmesartan was reintroduced on December 3, 2006 and continued until December 24, 2006 when the medication was again stopped. Records are incomplete but it also appears that the patient was hospitalized again in early December due to diarrhea, dehydration, hypokalemia and hypocalcemia, and it is noted that "Study medication was finally discontinued due to adverse events Diarrhea and Vomiting on December 30, 2006." This case is notable as it occurred in the closely monitored clinical trial setting and is both severe with multiple hospitalizations and has clear evidence of rechallenge. (Case SP-2006-003369.)

c) A 65 year old man had been treated with Benicar HCT for an unknown duration for hypertension. In May of 2007, he began to develop diarrhea. An antibiotic was prescribed for suspected infection without effect on his symptoms. Diarrhea continued and he reported being hospitalized 14 or 15 times for dehydration in 2007, with most hospitalizations lasting 4-5 days. He also experienced a 70 pound weight loss during this period. Weight loss was severe enough to warrant treatment with the

immunosuppressant 6-mercaptopurine, Total Parenteral Nutrition (TPN) and a surgically placed gastric feeding tube during the course of the year. After approximately seven months of symptoms, he had an endoscopy which diagnosed him with "gluten intolerance," likely due to presence of small intestinal villous atrophy. All of his medications were stopped late in 2007 and he was placed on a gluten free diet. At this time the diarrhea, lethargy and weight loss began to resolve. In October 2008, while still on a gluten free diet, the Benicar was restarted with return of diarrhea and weight loss. These symptoms slowly improved after Benicar was again stopped. It is notable that this patient has been given the diagnosis of Type 2 Refractory Celiac Disease, a severe and morbid prognosis and at the time of report was maintained on a gluten free diet despite rechallenge strongly suggesting olmesartan enteropathy rather than celiac disease as the cause of his severe malabsorption. (Case DSU-2009-002638.)

d) A 70 year old woman began taking olmesartan medoxomil for hypertension in 2005. On March 12, 2010 she developed nausea and vomiting. Later that month, on March 18, 2010 she presented to the emergency room for these symptoms and had an abdominal ultrasound which showed gallbladder sludge, a non-specific finding. She was admitted to the hospital at that time and was discharged 10 days later on March 28. She was then admitted to the hospital again for similar symptoms on April 7, 2010, during which time she had a cholecystectomy. Symptoms continued requiring placement of a central line and feeding

tube. Benicar was stopped and she was able to be discharged. A short time after discharge, Benicar was restarted on April 13, 2010. She was then hospitalized again from April 28 to May 3, 2010. While in hospital, Benicar was again discontinued and the patient was able to be discharged. After discharge the Benicar was restarted on May 7, 2010, after which symptoms returned. At that time the olmesartan was discontinued permanently and there has been no recurrence of gastrointestinal symptoms. In summary, this patient had recurrent hospitalizations and underwent surgery to remove her gallbladder for gastrointestinal symptoms which were related to olmesartan. (Case DSU-2010-02706.)

e) A 79 year old man had been treated with olmesartan starting in December of 2006. In November of 2009 he was diagnosed with clostridium difficile infection and was treated. In December, 2009, he began to experience weight loss and diarrhea. The patient underwent a very extensive evaluation for the etiology of these symptoms including exploratory laparotomy, PET scan, and CT scans. In the course of illness, and with 30 pounds unintentional weight loss, the patient's blood pressure became low and olmesartan was discontinued at an unclear point in time. Afterward it is noted that weight loss and diarrhea resolved by July of 2010, however when olmesartan was restarted in August 2010, diarrhea and vomiting recurred. Olmesartan was then discontinued and replaced with an alternate anti-hypertensive. (Case DSU-2012-02939.)

VI. THE MEDICAL LITERATURE PROVIDES EVIDENCE OF CAUSATION

32. The medical literature provides robust evidence that there is a causal connection between olmesartan and enteropathy with gastrointestinal symptoms and malabsorption. Different studies in a variety of populations have consistently found evidence of olmesartan related gastrointestinal toxicity.

33. The first reports of significant gastrointestinal disease in patients on olmesartan was in 2010 when Drs. Rubio-Tapia and Murray published a series of 30 patients with the rare disorder collagenous sprue and noted that about one third of the patients in this series were taking olmesartan.[43]

34. In 2012 Drs. Rubio-Tapia and Murray published a follow up paper describing 22 patients seen between 2008 and 2011 with enteropathy and chronic diarrhea, all of whom were taking olmesartan, between 10 and 40 mg per day for 6 months to 7 years before onset of symptoms.[38] The patients experienced diarrhea for a median of 19 months prior to suspension of olmesartan and all experienced at least some weight loss with a median of 18 kg, range 2 kg to 57 kg. 64% of the cohort had required at least one hospitalization and four required total parenteral nutrition. Improvement or complete remission was observed in all patients after cessation of olmesartan and 17 of 18 patients with a follow up small intestinal biopsy had histologic improvement. For patient safety and ethical reasons, there was no protocol for olmesartan rechallenge, however four patients in this series did resume olmesartan, all of whom reported recurrence of symptoms consistent with those experienced before the olmesartan was stopped.

35. In response to this report an article was subsequently published in the same journal in which the gastrointestinal treatment related adverse effects of the large pivotal 'Randomized Olmesartan and Diabetes Microalbuminuria Prevention (ROADMAP) study'

were presented.[44] This was the largest randomized controlled trial of olmesartan and included 2232 participants on olmesartan and 2215 on placebo followed for a median of 3.2 years. This analysis found no difference in the rate of intestinal or abdominal discomfort associated treatment related adverse effects including diarrhea and abdominal pain. It is notable that the most objective of these adverse events, 'weight decrease' was seen more frequently with olmesartan, although this event did not reach statistical significance. It is not surprising that this olmesartan enteropathy was not observed in the ROADMAP study initially published in 2011, as this study was underpowered for this adverse event based on number of patients included and length of follow up, did not include analysis of gastrointestinal effects as a primary or secondary endpoint, and involved diabetic patients leading to potentially confounding effects.

36. A second letter responding to the 2012 paper by Drs. Rubio-Tapia and Murray reports a patient with significant enteropathy, but with only mild anemia and upper gastrointestinal symptoms including reflux. Drs. Rubio-Tapia and Murray respond by noting that many of the patients in their series also had upper gastrointestinal symptoms which responded to olmesartan withdrawal and that there is "a spectrum of severity in olmesartan-associated-enteropathy."[45]

37. In 2013, more case reports were published documenting severe enteropathy and gastrointestinal illness in patients on olmesartan, all of whom achieved complete remission after cessation of this medication without further treatment.[37,46,47,48,49]

38. These reports and others directly reported to the FDA prompted the FDA Drug Safety Communication on 7-3-2013 detailing the olmesartan label change to include "intestinal

problems (sprue-like enteropathy) linked to blood pressure medication olmesartan medoxomil."[50] This statement advised health professionals:

- "Tell your patients to contact you if they develop severe, chronic diarrhea with substantial weight loss while taking an olmesartan-containing product, even if it takes months to years for symptoms to develop.
- If a patient develops these symptoms during treatment with olmesartan, other etiologies, such as celiac disease, should be investigated. If no other etiology is identified, olmesartan should be discontinued and another antihypertensive treatment started.
- Symptoms of sprue-like enteropathy may develop months to years after starting olmesartan."

Subsequent recommendations in the medical literature advised cessation of olmesartan in any patient with symptoms suggestive of enteropathy as an initial step, followed by further evaluation only if signs and symptoms do not remit.[51]

39. Also in 2013, a large series of 72 patients with enteropathy and blood tests negative for celiac disease was reported in a 10 year retrospective analysis of cases from a leading referral center.[37] In this series medication related enteropathy was the leading non-celiac diagnosis, making up 26% of cases and 16 of the 19 medication related enteropathy cases were patients on olmesartan. The remaining three cases were due to mycophenolate mofetil and methotrexate, both potent immunosuppressive medications. All cases had a clinical and/or histological response to cessation of olmesartan.

40. 2014 saw the publication of more than 30 articles on olmesartan enteropathy. The most notable of these was the publication in abstract form of a French nationwide cohort study, published in full in 2015.[39] This study made use of the French National Health Insurance claim database to evaluate all adult patients initiating ARB or ACEI between 1 January 2007 and 31 December 2012 with no prior hospitalization for intestinal malabsorption or treatment for celiac disease. A total of 4,552,130 patients initiating ARB or ACEI treatment

were included. A total of 4,546,680 patients corresponding to 9 129 149 person-years were included. In this cohort, 218 hospitalizations for intestinal malabsorption were observed. The overall rate was 5.6 per 100,000 person years in the olmesartan group compared to 2.4 per 100,000 person years in the ACEI group and 1.8 per 100,000 person years in the non-olmesartan ARB group. This difference was highly statistically significant ($p<0.0001$). Additionally this study found that risk of hospitalizations for intestinal malabsorption increased significantly with time for the olmesartan cohort, from 2.65 hospitalizations per 100,000 person years with less than 1 year of exposure to 6.71 hospitalizations per 100,000 person years with 1-2 years of exposure to 8.86 hospitalizations per 100,000 person years with 2 or more years of exposure to olmesartan. In comparison, there was a trend to decreased risk with time for the ACEI and non-olmesartan ARB cohorts, in other words, only olmesartan was found to be associated with hospitalization for intestinal malabsorption. This is a very robust study demonstrating an increased risk of hospitalizations for intestinal malabsorption with olmesartan. At the same time, as it only looked at hospitalizations, it is very likely an underestimate as the authors note "it is unlikely that all cases of olmesartan-associated enteropathy were captured by hospital diagnoses of intestinal malabsorption and coeliac disease. It is likely that milder forms also exist."[39]

41. Along with multiple other case reports of olmesartan enteropathy, a number of other more systematic studies of this condition were published in 2014.[3,52,53,54,55,56,57,58,59,60,61,62,63,64,65,66,67,68,69,70]

- A study which surveyed French gastroenterologists for any suspected case of diarrhea related to ARB use reported 48 cases, of which 47 were associated with olmesartan and one was associated with irbesartan.[52] Of these 48 cases, data was

available for 40 patients. Of these 40 patients, one was on irbesartan, one had normal duodenal biopsies, and two didn't have any biopsies, and thus were excluded from analysis, leaving 36 patients. Of these 36, 32 had documented enteropathy while four had significant malabsorption with diarrhea and dehydration but without enteropathy on biopsy and 31 patients required hospitalization. This series also reported 9 cases in which cessation of olmesartan led to remission of symptoms followed by recurrence of symptoms with resumption of olmesartan.

- A case control study done out of a single center looked back at 2088 patients undergoing EGD and 12,428 patients undergoing colonoscopy. This study did not find any association between patients on olmesartan and the reported symptom of diarrhea at the time of endoscopy, however only a total of 105 patients were exposed to olmesartan which as the authors note "small prevalence of use of olmesartan (0.7%-1%) among study patients, limiting the power of this analysis."[58]

- A systematic review identified 11 publications totaling 54 patients, almost all of whom had diarrhea and weight loss on olmesartan. All patients' symptoms resolved upon discontinuation of this medication. Other common symptoms included fatigue (56%), nausea and vomiting (45%), and abdominal pain (37%). This study also noted that 98% of patents had villous atrophy on small intestinal biopsy, increased collagen in the small intestine was seen in 33% of patients, 45% were anemic and 39% had low albumin levels. 72% were positive for HLA DQ2 or DQ8 while celiac antibody tests were universally negative.[3]

- A study in which patients undergoing outpatient upper endoscopy for abdominal pain, 20 of whom were on olmesartan, 20 of whom were non non-olmesartan ARBs and 40 of whom were not on any of these medications, were reviewed for enteropathy. This study found that 50% of patients on olmesartan had one or more features of enteropathy, compared to 20% of matched controls. Conversely, non-olmesartan ARBs had a similar frequency of enteropathy compared to matched controls. This study suggests that there is likely to be a spectrum of intestinal injury seen with olmesartan, much of which does not result in symptoms severe enough to require hospitalization.[70]

42. Studies in 2015 continued to document further case reports and case series of olmesartan enteropathy, and in addition several further studies of the pathogenesis and histological presentation were published.[2,30,34,35,39,70,71,72,73,74,75,76,77,78,79,80,81,82,83,84,85,86,87,88]

- A group from the Mayo Clinic comprised of many of the authors who initially reported on olmesartan enteropathy, published a study of the pathogenesis of this condition. This study utilized intestinal biopsies taken from patients with olmesartan enteropathy on and off of the medication to evaluate the immune mechanisms of olmesartan related inflammation. This paper demonstrates that epithelial cells respond to olmesartan acid by increasing the expression of IL-15 and disruption of the key tight junction protein ZO-1 with a related increase in cytotoxic CD8+ cells. They suggest that a potential unifying theory for olmesartan enteropathy is that patients in certain circumstances are unable to control the increased IL-15 expression induced by olmesartan medoxomil, and as a result develop enteropathy.[30]

- A study from Sweden evaluated the link between angiotensin receptor blockers and enteropathy by linking nationwide histopathology data and the Swedish Prescribed Drug Register.[88] This study evaluated the risk of non-olmesartan angiotensin receptor blockers use and the risk of ACE inhibitor use in 2,933 individuals with enteropathy and 14,571 controls. The authors reported that there was no relationship between enteropathy and either ARB or ACE inhibitor use, a finding which suggests that olmesartan enteropathy is largely drug specific and not a class effect.

- A further paper looked at the histological spectrum of olmesartan enteropathy as described in the literature to date. The authors reported that 92 of 100 individuals (92%) had total or partial villous blunting while 5% had normal villous architecture. Increased IELs were reported in 61 of 100 biopsies and subepithelial collagen thickening was reported in 22 of 100 biopsies. Variable degrees of chronic inflammation, acute inflammation, and increased eosinophils can be seen. Importantly, microscopic involvement of parts of the gastrointestinal tract other than the duodenum may be seen. Findings may include ulcers, lymphocytic and/or collagenous gastritis and changes in the colon that can be mistaken for lymphocytic or collagenous colitis.[2]

43. 2016 saw more publications related to gastrointestinal side effects of olmesartan.[89,90,91,92,93,94,95,96,97]

- A regional Spanish registry study reported on 20 patients with olmesartan enteropathy.[91] In this cohort, 14 patients (87.5%) required hospitalization, three patients had inflammation of the stomach and ten had inflammation of the

colon. In addition, lupus like conditions developed in three of the patients with arthritis and high titer auto-antibodies including anti-nuclear antibody (ANA). This lupus-like condition resolved with the gastrointestinal symptoms after withdrawal of olmesartan.

- An intriguing paper from one of the top celiac disease groups in Europe also reported two cases of olmesartan enteropathy with onset after an acute infection.[90] This phenomenon of post-infectious onset of immune mediated disease has been documented in celiac disease, microscopic colitis as well as diseases outside the intestine including neurologic disease and kidney disease and supports the concept that olmesartan, under the right genetic and environmental pressures, can trigger a drug dependent immune mediated enteropathy.

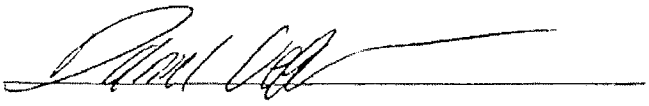
VII. SUMMARY OF MEDICAL LITERATURE REGARDING ASSOCIATION OF OLMESARTAN AND ENTEROPATHY

44. The medical literature addressed above provides strong evidence that olmesartan causes enteropathy of varying severity, with associated gastrointestinal symptoms which also may range from very severe requiring prolonged hospitalization and intravenous nutritional support, to relatively mild. Taken as a whole, the literature establishes a temporal relationship between duration of exposure to olmesartan and risk of enteropathy and intestinal malabsorption with significant variability in time to onset of olmesartan enteropathy. While no formal prospective studies of olmesartan rechallenge have been conducted, and are unlikely to be conducted due to ethical issues, numerous case reports attest that patients with olmesartan improve with cessation of this drug and relapse promptly if re-exposed to olmesartan. While understanding of the pathophysiology of olmesartan enteropathy is evolving, this appears to be

largely agent specific and does not involve other ARBs. Olmesartan enteropathy also appears to be an immune mediated phenomenon where olmesartan binds or otherwise upregulates IL15 and triggers CD8 mediated cytotoxicity.

45. Based on these data, my current clinical practice is to evaluate all patients presenting with gastrointestinal symptoms or signs of malabsorption for olmesartan or other potential causative agents. Olmesartan is discontinued at this time and if symptoms and malabsorption resolves, the diagnosis of olmesartan enteropathy can be made. There is no recommendation for rechallenge, or endoscopy to either confirm enteropathy or confirm resolution of enteropathy in patients with a clear and adequate clinical response. Alternatively, some patients are recognized to have enteropathy at endoscopy done for symptoms which have not been recognized to be potentially related to olmesartan enteropathy. All patients with a new diagnosis of enteropathy should be evaluated for olmesartan use and this medication discontinued when applicable. To proceed with extensive evaluation while continuing olmesartan places the patient at significant additional risk due to delay in instituting the definitive effective treatment of olmesartan withdrawal, as well as the risk and cost of unnecessary testing. My advice is noted in recent articles including in the conclusion of a recent review of olmesartan enteropathy in the Annals of Internal Medicine, "All patients should be warned to advise their physician and stop the drug if they develop any diarrhea or weight loss on olmesartan"[87] and in Cartee and Murray's *Sprue-like Enteropathy Associated with Olmesartan,* "It would seem reasonable to hold olmesartan much earlier in the natural history of the illness rather than assuming other diagnosis first in order to limit worsened symptoms leading to nutritional deficiencies and requiring a greater level of care and more extensive diagnostic evaluations."[51]

46. I reserve the right to supplement this expert report based on new information.



11-24-16
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FIGURE 1

Figure 1. Healthy Small Intestinal Lining Photomicrograph and Illustration

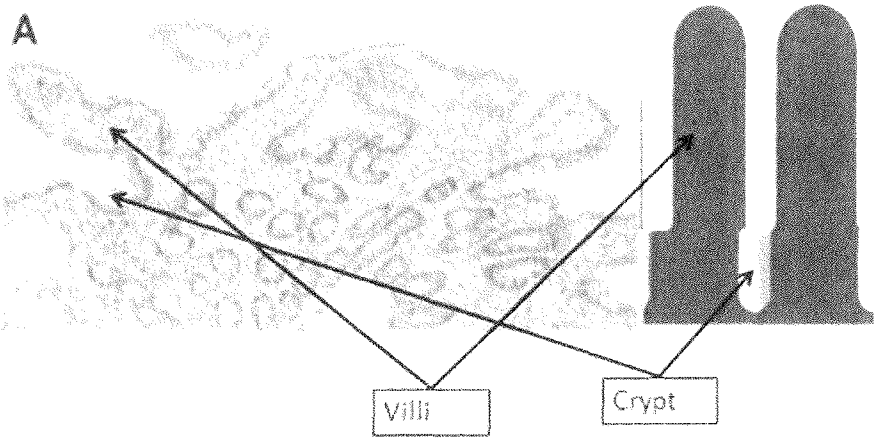
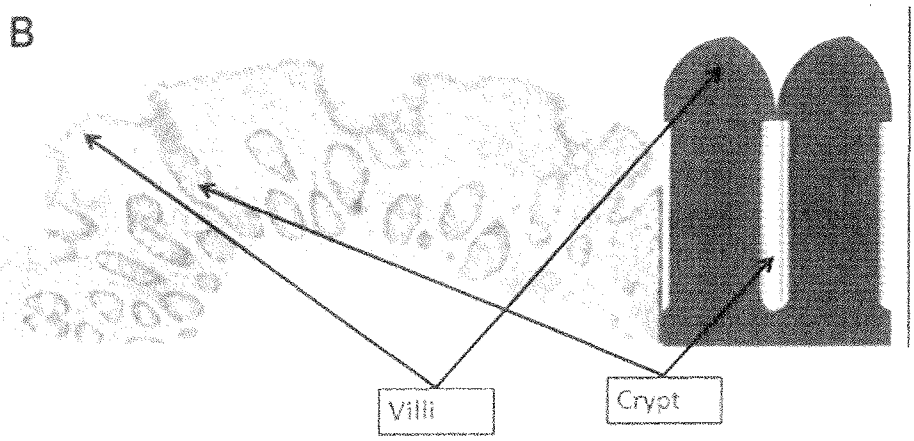


FIGURE 2

Figure 2. Small Intestinal Lining with Villous Atrophy/Enteropathy Photomicrograph and Illustration



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[97] Olmesartan: sprue-like enteropathy. Prescrire Int 2016;25:130-1.

2015 Fee Schedule and Contact Information For Medical Legal Case Reviewing

Daniel Leffler, MD, MS
Director of Quality Improvement
Research Director @ The Celiac Center
Division of Gastroenterology
Associate Director of Research and Quality
Department of Medicine
Beth Israel Deaconess Medical Center
Associate Professor of Medicine
Harvard Medical School

Address: 15 Clinton Place
Newton, MA 02459

Phone: 781-608-5918 (Home office)
617-667-1272 (BIDMC, Boston office)

Fax: 617-667-8144 (BIDMC, Boston)

Email: dleffler@bidmc.harvard.edu

Important Note: Please allow 2-4 weeks for completion of reviews.

Fee Schedule:

$475 per hour for reviewing, report writing, and conferences
- 3 hour retainer fee prior to initiating review

$750 per hour (or part thereof) for depositions
- 2 hour minimum, payable in advance

$250 per hour plus expenses for travel to/from depositions or conferences
- 1 hour minimum

$5,000.00 + expenses for court appearances
+ $1500 per half day for additional travel/wait days
- Payable in full, 2 weeks in advance of the scheduled court date

Late cancellation fees (for events cancelled or postponed less than 14 days in advance of scheduled date
- Depositions in Greater Boston area – $1500.00
- Depositions outside Greater Boston - $2,500.00

PRIOR TESTIMONY DURING THE PAST 3 YEARS

Ronda Orozco v. Boston Scientific Corporation d/b/a Mansfield Scientific Inc., Civil Action No. MICV2012-03068, Superior Court, Middlesex

Deposition for Defendant

Harvard Medical School Curriculum Vitae

Date Prepared: 10/20/16

Name: **Daniel Alexander Leffler, MD, MS**

Office Address: Beth Israel Deaconess Medical Center
Dept. of Gastroenterology
330 Brookline Ave., E/Stoneman-385
Boston, MA 02215

Home Address: 15 Clinton Place
Newton, MA 02459

Work Phone: 617.667.1272

Work Email: dleffler@caregroup.harvard.edu

Work FAX: 617.667.8144

Place of Birth: Boston, Massachusetts

Education

09/92-06/96	B.S.	Biochemistry	Colorado College, CO
09/97-09/98	M.S.	Nutrition	Columbia University, NY
09/98-07/02	M.D.	Medicine	Ben Gurion University, Israel/ Columbia University, NY

Postdoctoral Training

07/02-06/03	Intern	Medicine	Beth Israel Deaconess Medical Center
07/03-06/05	Resident	Medicine	Beth Israel Deaconess Medical Center
07/05-06/06	Research Fellow	Gastroenterology	Beth Israel Deaconess Medical Center
07/06-06/08	Clinical Fellow	Gastroenterology	Beth Israel Deaconess Medical Center

Faculty Academic Appointments

07/08-05/09	Instructor	Medicine	Harvard Medical School
06/09-9/13	Assistant Professor	Medicine	Harvard Medical School
09/13-Present	Associate Professor	Medicine	Harvard Medical School

Appointments at Hospitals/Affiliated Institutions

07/08-present	Attending Physician Medicine (Gastroenterology)	Beth Israel Deaconess Medical Center
07/10-07/12	Research Specialist Physician	Fenway Community Health

Other Professional Positions

07/2016-present	Medical Director	Gastrointestinal Therapeutic Area	Takeda Pharmaceuticals

Major Administrative Leadership Positions

Local

07/05-06/08	Clinical and Research Coordinator, Celiac Center	Beth Israel Deaconess Medical Center
07/08-Present	Director of Clinical Research, Celiac Center	Beth Israel Deaconess Medical Center
07/10-Present	Director, Celiac Center Clinical and Research Fellowship	Beth Israel Deaconess Medical Center
04/11-06/2016	Director of Quality Improvement, Division of Gastroenterology	Beth Israel Deaconess Medical Center
04/11-06/2016	Associate Director of Research for Quality Improvement, Department of Medicine	Beth Israel Deaconess Medical Center
07/12-06/2016	Clinical Research Director, Gastroenterology Fellowship Program	Beth Israel Deaconess Medical Center
08/12-06/2016	Course Co-Director, Patient Safety Core Faculty	Beth Israel Deaconess Medical Center
07/15-07/2016	Associate Firm Chief, Blumgart Firm	Beth Israel Deaconess Medical Center

Committee Service *(Member except where noted)*

Local

2008- 2016	Medical Peer Review Committee Department of Medicine	Beth Israel Deaconess Medical Center
2009-2016	Interventional Procedures Committee	Beth Israel Deaconess Medical Center
2009-2016	Gastrointestinal Hemorrhage Task Force	Beth Israel Deaconess Medical Center
2011-2016	Department of Medicine QI Leadership Council	Beth Israel Deaconess Medical Center
2012-2015	Colorectal Cancer Screening Advisory Committee	CRICO-Risk Management Foundation
2012-2016	Patient Safety Core Faculty, Department of Medicine	Beth Israel Deaconess Medical Center
2012-2016	Founding member of Center for Healthcare Delivery Sciences	Beth Israel Deaconess Medical Center
2014-2016	External Peer Review for Gastroenterology	Beth Israel Deaconess Network
2015-2016	Research Review Committee Center for Primary Care at Harvard Medical School	Harvard Medical School
2016	Poster Review Committee Soma Weiss Student Research Day	Harvard Medical School
2016-Present	Chairman; Colorectal Cancer Screening Advisory Committee	CRICO-Risk Management Foundation

National and International

2010-Present	Medical Advisory Board Healthy Villi Celiac Advocacy Group

2010-Present	Medical Advisory Board Beyond Celiac (www.celiaccentral.org)
2010-2014	Collaborator/Founding Member Oslo Group on Standards and Definitions for Celiac Disease and Related Disorders
2012-2014	Secretary/Founding member North American Society for the Study of Celiac Disease (www.nassed.org)
2013-Present	Medical Advisory Board Celiac Disease Foundation (www.celiac.org)
2013-Present	Digestive Diseases Week Abstract Review Committee American Gastroenterological Association
2014-Present	Scientific Grant Review Committee Italian Celiac Association 'Associazione Italiana Celiacia'
2014-2015	Steering Committee Member for the FDA Gastrointestinal Regulatory Endpoints and Advancement of Therapeutics Conference on Celiac Disease

Professional Societies *(Member except where noted)*

2005-Present	American Gastroenterological Association (AGA)
2005-Present	American College of Gastroenterology
2009-2011	AGA Press Advisory Board
2010-2016	Intestinal Diseases Section, AGA Institute Council
2010-Present	AGA Media Speakers' Bureau
2011-2015	AGA Academy of GI and Liver Educators Advisory Board
2016-Present	Councilor, AGA Institute, Intestinal Diseases Section

Editorial Activities

Ad hoc reviewer

- Alimentary Pharmacology and Therapeutics
- American Journal of Clinical Nutrition
- American Journal of Epidemiology
- American Journal of Gastroenterology
- American Journal of Physiology - Gastrointestinal and Liver Physiology
- Annals of Internal Medicine
- Annals of Neurology
- Appetite
- Archives of Internal Medicine
- British Medical Journal; Point-of-Care Physicians Reference Database
- British Journal of Nutrition
- Clinical Chemistry and Laboratory Medicine
- Clinical Infectious Diseases
- Diabetologia
- Digestive Diseases and Sciences

- European Journal of Clinical Investigation
- Expert Opinion on Drug Discovery
- Expert Review of Gastroenterology and Hepatology
- Gastroenterology
- Gastrointestinal Endoscopy
- Journal of Clinical Gastroenterology
- Journal of the American College of Nutrition
- Journal of the American Medical Association
- Journal of Medical Internet Research
- Journal of Pediatrics
- New England Journal of Medicine
- Therapeutic Advances in Gastroenterology

Other Editorial Roles

2010-Present	Editorial Board	Clinical Gastroenterology and Hepatology
2011-2013	Editorial Board	BMC Gastroenterology
2013-Present	Editorial Board	World Journal of Gastroenterology

Honors and Prizes

1994	Undergraduate Research Grant, Howard Hughes Institute
2006	Presidential Poster Award, American College of Gastroenterology
2006	AstraZeneca Senior Fellow Abstract Award, American College of Gastroenterology
2006	Program in Clinical Research Effectiveness, Harvard School of Public Health
2007	AstraZeneca Senior Fellow Abstract Award, American College of Gastroenterology
2010	PCIR Junior Investigator Laboratory Support Award, Harvard Catalyst
2012	Irving W. and Charlotte F. Rabb Prize for Gastroenterology Research
2013	Dvorak Young Investigator Award for Health Services Research
2013	Journal Author Excellence Award: American Society for Healthcare Risk Management
2015	Nezam Afdhal Outstanding Achievement in the Field of Excellence Award for Innovation in Gastroenterology

Teaching Awards

2008	Excellence in Tutoring Award, HMS Academy Center for Teaching and Learning
2010	Nominee, Excellence in Mentoring Award, HMS Office of Diversity and Community Partnership
2013	Nominee, Excellence in Mentoring Award, HMS Office of Diversity and Community Partnership
2014	Mentorship of Resident Research Award, Beth Israel Deaconess Medical Center

Report of Funded and Unfunded Projects

Past

2008-2010 Optimizing Safety in Ambulatory Procedural Care: Risk Informed Interventions (PI, Dierks, Meghan)
AHRQ R18 HS017907-01
Co-Investigator
My role was to provide guidance on risk areas in gastrointestinal endoscopy and assist with program design and evaluation including statistical methodology.

2006-2010 Assessing Gluten Free Diet Adherence in Adults with Celiac Disease (PI, Kelly, Ciaran)
Celiac Sprue Association
Co-Investigator
Major goal: to develop ways to measure dietary adherence in celiac disease as well as patient reported outcomes assessing symptoms and quality of life. I was responsible for study design, patient recruitment, statistical assessment and publication of results.

2008-2010 Development of a Long Term Follow-Up Alerting System for Integration into Electronic Medical Records (PI, Aronson, Mark)
CRICO/Risk Management Foundation
Co-Investigator
Major goal: to develop a framework for integrating reminder systems for patients needing repeat testing in the more distant future (>1 year). I was responsible for program design, evaluation, running patient and physician focus groups, liaising with IT specialists and assessing system performance.

2008-2010 Unrestricted Research Grant to Evaluate Clinical Outcomes in Celiac Disease
Alvine Pharmaceuticals (PI, Kelly, Ciaran)
Co-Investigator
Major goal: to develop and validate much needed non-invasive measures of celiac disease activity. I was responsible for study design, execution and evaluation.

2009-2010 Schwartz Center Connections (PI, Stanzler, Marjorie)
Schwartz Center and CRICO-RMF
Co-Investigator
Major goal: Working with a multi-disciplinary team led by the Schwartz Center and CRICO-RMF, this project aimed to identify and codify communication pathways between healthcare providers. Specific attention was paid to issues leading to miscommunication and adverse patient outcomes.

2008-2010 Eliminating Healthcare Disparities through Education: A Universal Medical School Program (PI, Shields, Helen)
Interfaculty Collaboration of Harvard Medical School, Harvard Business School and Harvard Graduate School of Education
Co-Investigator
Major Goal: To create a model for reducing health care disparities through the development of a longitudinal, integrated, tested educational program across all four years of medical school.

2011-2013 Clinical Utility of HLA Typing in Suspected Celiac Disease
Prometheus Laboratories
PI
Major goal: to evaluate use of HLA testing in celiac disease and to provide evidence-based guidelines for HLA testing and interpretation of results.

2012-2013 Evaluation of Mesalamine's Therapeutic Potential in Celiac Disease

Shire Pharmaceuticals
PI ($111,129)
Major goal: to evaluate the in vitro efficacy of mesalamine to reduce celiac disease related intestinal inflammation

2009-2014 Biomarkers of Celiac Disease Activity During Gluten Challenge
NIH K23 DK082619
PI ($931,230)
Major goal: As PI on this 5-year Mentored Patient-Oriented Research Career Development Award, our goal is to develop patient reported outcomes and non-invasive biologic markers to evaluate celiac disease activity.

2009-2014 Campaign to Improve Awareness of Celiac Disease Among Primary Care Physicians (PI, Kelly, Ciaran)
Sydney E. Frank Foundation
Co-Investigator ($250,000)
Major goal: to develop tools to increase awareness and diagnosis rates of celiac disease in primary care practices. I assist with study design, content of informational module, and assessment of intervention efficacy.

2013-2014 Moderate Sedation – the final frontier for quality in procedural areas. - A multidisciplinary team based approach towards continuous improvement.
BIDMC Center for Healthcare Delivery
Co-Principal Investigator ($42,000)
Major goal: to address the lack of moderate sedation benchmarks and to ensure that our patients can expect the safest and highest quality moderate sedation possible, and to monitor the effects of medication shortages and compare the costs and benefits of anesthesia administered deep sedation.

Current

2013-2015 Validation of Peripheral Microparticles as Novel Biomarkers of Celiac Disease Activity
NIH R03DK095937
PI ($100,000)
Major Goal: The identify accurate non-invasive measures of celiac disease activity, which would be of great value in clinical practice, are prerequisite to the testing of new treatment modalities, and may offer insight into disease pathogenesis

2013-2015 Referral Management
CRICO-RMF
Co-investigator.($393,988)
Major Goal: The aim of this project is to develop and pilot systems for improved monitoring of patient referrals with the goals of improving adherence and reducing medical-legal consequences.

2014-2017 Measuring and Improving Colonoscopy Quality Using Natural Language
NIH-R01CA168959-04
Co-Investigator ($905,880)
Major Goal: To develop a natural language processing system to automate assessment of colonoscopy quality and to use these data to evaluate physician factors which influence quality and assess the ability of performance metrics to lead to quality improvements.

Pending Risk & Outcomes from Screening & Treating Celiac Disease in T1D Adults: an RCT
NIH R01DK107857-01
PI ($1,949,080)
Major Goal: This prospective, randomized controlled trial will provide rigorous data on the potential benefits of screening adults with T1D for celiac disease in the United States and on the outcomes associated with celiac disease screening initiatives on which to base health policy and medical guidelines.

Current Unfunded Projects

2008-Present Timing of Colonoscopy and Polyp Detection Rate is a project that assesses the influence of time of day in polyp detection to assess for endoscopist fatigue similar to what has been shown in surgical literature. (Co-investigator)

2008-Present Outcomes of Complicated Celiac Disease is a project to assess outcomes and medication usage of patients with non-responsive celiac disease, refractory celiac disease and celiac crisis. (Co-investigator) .

Report of Local Teaching and Training

Teaching of Students in Courses

2005 Examiner, Objective Structured Clinical Exam Training
Harvard Medical Exchange Students
Harvard Medical School
3 hrs. contact time

2005 Fourth Year Medical Student Clinical Skills Tutorial
HMS Medical Students
Harvard Medical School
6 hrs. contact time

2005-2008 Tutor, Gastrointestinal Pathophysiology Course 708.0 *Human Systems/Module IIA*
HMS medical students
Harvard Medical School
Three, 2-hr. sessions per week for 4 weeks

2006-2012 Board review Sessions, Gastrointestinal Pathophysiology 708.0, *Human Systems/Module IIA*
HMS medical students
Harvard Medical School
2, 1-hr. sessions per year

2009-Present Pathology Lab Co-Instructor, Gastrointestinal Pathophysiology 708.0, *Human Systems/ Module IIA*
HMS medical students
Harvard Medical School
Three, 2-hr. sessions per week for 4 weeks

Formal Teaching of Residents, Clinical Fellows and Research Fellows (post-docs)

2007, 2010, 2013 Update on Celiac Disease
Medical Interns and residents

Beth Israel Deaconess Medical Center
1 hr. lecture

2007-Present Advanced Diagnosis and Management of Celiac Disease
15 Clinical Gastroenterology Fellows
Beth Israel Deaconess Medical Center
1-hr didactic sessions, annually

2012-Present Annual Fellows QI and Safety Symposium
40 Clinical Medicine fellows (all subspecialties)
Beth Israel Deaconess Medical Center
3-hr didactic sessions, 4 x annually

Clinical Supervisory and Training Responsibilities

2008-Present Consult Attending in Gastroenterology, Beth Israel Deaconess Medical Center
4 weeks per year, 3-4 hours per day

2008-Present Endoscopy Training for Fellows, Beth Israel Deaconess Medical Center
One day per month, 8 hours per day

2012-Present Ad hoc guidance for senior medical residents and gastroenterology fellows working on quality improvement and patient safety projects.

Laboratory and Other Research Supervisory and Training Responsibilities

2006-Present Supervision of residents and research assistants working on clinical research projects.

2008-2013 I mentor medical residents at BIDMC in an ongoing case-control design project to assess the influence of ethnicity on risk and severity of Clostridium difficile infection.

2009-2013 I mentor medical residents at BIDMC on a survey based project to evaluate patient perceived burden of the gluten free diet in celiac disease in comparison to treatments for a variety of other medical conditions. This study will also assess the influence of socioeconomic status and educational level on burden of disease and reported treatment adherence.

Formally Supervised Trainees

2008-2009 **Shailaja Jamma, MD** / Research Fellow
Received a research award from the American College of Gastroenterology and published multiple abstracts and manuscripts. Completed gastroenterology fellowship at University of Chicago. Currently on staff at Gastrointestinal Care Consultants Houston, TX.

2010-2011 **Kumar Pallav, MD** / Celiac Clinical/Research Fellow
Supervised the planning and execution of multiple clinical research projects as well as patient care. Completed gastroenterology fellowship at University of Mississippi. Currently on faculty at University of Mississippi

2011-2013 **Toufic Kabbani, MD** / Celiac Clinical/Research Fellow
Supervised the planning and execution of multiple clinical and translational research projects, resulting in multiple published manuscripts and presentations at national meetings. Also formally supervised patient care activities. Currently in gastroenterology fellowship at University of Pittsburgh Medical Center

2012-2013 **Rohini Vanga, MD** / Celiac Clinical/Research Fellow
Supervised the planning and execution of multiple clinical and translational research projects. Also

formally supervised patient care activities. Currently in gastroenterology fellowship at Baylor University

2013-2014 **Themaiah Theetira, MD** / Celiac Clinical/Research Fellow
Supervised the planning and execution of multiple clinical and translational research projects. Also formally supervised patient care activities. Currently in gastroenterology fellowship at University of California, Fresno

2014-2016 **Dharmesh Kaswala, MD** / Celiac Clinical/Research Fellow
Supervised the planning and execution of multiple clinical and translational research projects. Also formally supervised patient care activities. Starting gastroenterology fellowship at California Pacific Medical Center, July 2016

2014-2016 **Gopal Veeraghavan, MD** / Celiac Clinical/Research Fellow
Supervised the planning and execution of multiple clinical and translational research projects. Also formally supervised patient care activities. Starting gastroenterology fellowship at University of California, Fresno, July 2016

2015-2016 **Satya Kurada, MD** / Celiac Clinical/Research Fellow
Supervised the planning and execution of multiple clinical and translational research projects. Also formally supervised patient care activities.

2013-2015 **Adelina Hung, MD** BIDMC Medical Resident Supervised the planning and execution of multiple clinical and quality improvement projects. Currently in gastroenterology fellowship at Yale.

2014-2016 **Manida Wungjiranirun, MD** BIDMC Medical Resident. Supervised the planning and execution of multiple clinical and quality improvement projects. Starting gastroenterology fellowship at Brown University, July 2016

2015-2016 **Katherine Germansky, MD** BIDMC gastroenterology fellow. Supervised the planning and execution of multiple clinical and quality improvement projects. Starting on faculty at Beth Israel Deaconess Medical Center, July 2016.

2015-2016 **Sarah Shannahan, MD** BIDMC Medical Resident. Supervising celiac disease research projects and review articles.

2015-2016 **Laurie Grossberg, MD** BIDMC gastroenterology Fellow. Supervising quality improvement research projects looking at colonoscopy safety.

Local Invited Presentations *No presentations below were sponsored by outside entities*

2007-2009 Gastrointestinal Bleeding Module, Bedside Emergencies Conference
Clinical RNs
Beth Israel Deaconess Medical Center, Boston, MA (1-hr session, 3 x annually)

2010 Celiac Disease: A Modern Understanding of an Ancient Disease / Clinical Grand Rounds
Brigham and Woman's Hospital, Boston, MA

2010 Celiac Disease: Lessons from an Ancient Disorder / Gastroenterology Grand Rounds
Massachusetts General Hospital, Boston, MA

2011 Clinical Crossroads: Celiac Disease / Medical Grand Rounds
Beth Israel Deaconess Medical Center, Boston, MA

2011 Celiac Disease: The Usual and Unusual
Brigham Update in Medicine - Partners Healthcare
Boston, Ma

2011 Celiac Disease: Modern Lessons From an Ancient Disease

Updates in Gastroenterology Lecture series
Beth Israel Deaconess Medical Center, Boston, Ma

2011, 2012 Cross-Cultural Care in the Pre-Clinical Years / Use of Case Triggers and Vignette Writing
Healing Health Care Disparities through Education: An Interactive Faculty Development Program, Harvard Medical School, Boston, MA

2011 Update in Celiac Disease / Allergy/Immunology Grand Rounds
Massachusetts General Hospital, Boston, MA

2011 Celiac Disease and Infertility / Grand rounds
Boston IVF / Division of Reproductive Endocrinology and Infertility
Beth Israel Deaconess Medical Center, Boston, MA

2011 Celiac Disease: Modern Lessons From an Ancient Disease / Grand Rounds
Beth Israel Deaconess Needham, Needham, MA

2012 Celiac Disease: Modern Lessons From an Ancient Disease / Rheumatology Grand Rounds
Brigham and Women's Hospital, Boston, MA

2012 Celiac Disease: Update for PCPs
Meet the Professor
Office Practice of Primary Care Medicine Symposium
Brigham and Women's Hospital, Boston, MA

2012 Celiac Disease: Modern Lessons From an Ancient Disease / Grand Rounds
Joslin Diabetes Center, Boston, MA

2012 Celiac Disease: Endocrine Aspects / Endocrinology Grand Rounds
Children's Hospital Boston, Boston, MA

2013 Celiac Disease: Lessons from the Adult World / Gastroenterology Grand Rounds
Children's Hospital Boston, Boston, MA

2014 Course Director: Academic Achievement in Quality Improvement. Beth Israel Deaconess Medical Center, Boston, MA

Report of Regional, National and International Invited Teaching and Presentations

Invited Presentations and Courses

Regional *Those presentations below sponsored by outside entities are so noted and the sponsor is identified*

2008 Celiac Disease Update / Invited speaker
A Core Curriculum In Primary Care Medicine
Boston University School of Medicine

2009 The Modern Face of Celiac Disease / Invited Keynote speaker
New England Society of Gastroenterology Nurses and Associates (NESGNA)
Annual Educational Conference, Boston, MA

2010 An Update on Celiac Disease Diagnosis and Treatment / Medical Grand Rounds
Sturdy Memorial Hospital
Attleboro, Ma

2012 Celiac Disease: Protean Manifestations/Invited speaker
Update in Internal Medicine, Beth Israel Deaconess Medical Center
Boston, MA

2014 Celiac Disease
Gastroenterology Grand Rounds Massachusetts General Hospital
Boston, MA

2014	Update on Celiac Disease Harvard University Health Services Boston, MA
2015	Review of Gastroenterology / Course instructor ACP Review Course American College of Physicians Boston, MA
2015	Colorectal Cancer Screening: Current Recommendations and Controversies Best Practices CRICO-RMF Boston, MA
2016	Celiac Disease and Gluten Sensitivity: Similarities and Differences Update in Internal Medicine, Beth Israel Deaconess Medical Center Boston, MA

National *Those presentations below sponsored by outside entities are so noted and the sponsor is identified*

2008	Patient Reported Outcomes in Celiac Disease / Invited speaker Clinical Trial Outcomes in Celiac Disease (Alba Pharmaceuticals) New York City
2009	Meeting the Needs of Adults with Celiac Disease / Invited speaker Food and Nutrition Conference and Expo American Dietetic Association Denver, CO
2009	Safety, Tolerability and Effects On Intestinal Permeability of Larazotide Acetate in Celiac Disease: Results of a Phase IIB 6-Week Gluten-Challenge Clinical Trial (Abstract) Digestive Diseases Week, American Gastroenterological Association Chicago, IL
2010	Quality of Life and Patient Reported Outcomes in Celiac Disease / Invited speaker Development of Therapies for Celiac Disease Columbia University New York City
2010	1) Focused Clinical Update: Celiac Disease / Invited speaker 2) Meet the Professor Lunch: Refractory Celiac Disease and Diet Adherence 3) An Update on Celiac Disease Diagnosis and Treatment / Session Chair 4) Diagnosis and Highly Effective Medical Management of Celiac Disease / Session Chair Digestive Diseases Week, American Gastroenterological Association New Orleans, LA
2010	An Update on Celiac Disease Diagnosis and Treatment / Invited speaker Medical and Surgical Advances in Gastroenterology Lee Memorial Health System, Fort Meyers, FL
2010	Celiac Disease: Protean Manifestations / Invited speaker PRIMED Update in Primary Care (PRIMED) Boston, MA
2011	Refractory Celiac Disease Research Forum / Session Chair Digestive Diseases Week, American Gastroenterological Association Chicago, IL

2011	1) Celiac Disease: Protean Manifestations / Invited speaker 2) Clostridium Difficile Infection: Not Just a Hospital Problem / Invited speaker PRIMED Update in Primary Care (PRIMED) Anaheim, CA
2011	Celiac Disease: Important and Often Missed / Invited speaker Author in the Room, IHI/JAMA Boston, MA
2011	PROs for Celiac Disease: Measuring What Patients Care About / Gastroenterology Grand Rounds Columbia University, NYC
2012	Celiac Disease: Modern Lessons From an Ancient Disease / Grand Rounds Pinnacle Health System/Harrisburg Hospital Harrisburg, PA
2012	1) Clostridium Difficile Infection: Not Just a Hospital Problem / Invited speaker 2) Celiac Disease: Protean Manifestations / Invited speaker PRIMED Update in Primary Care (PRIMED) Fort Lauderdale, Fl
2012	AGA Academy of Educators Plenary Session/ Invited speaker Digestive Diseases Week, American Gastroenterological Association San Diego, CA
2012	Review of Gastroenterology / Course instructor ACP Review Course American College of Physicians Boston, MA
2012	Gastroenterology Ask the Expert PRIMED Update in Primary Care (PRIMED) Boston, MA
2013	Visiting Professor/Gastroenterology Grand Rounds Northwestern Medical Center Chicago, IL
2013	Review of Gastroenterology / Course instructor ACP Review Course American College of Physicians San Francisco, CA
2013	1) Celiac Disease: Protean Manifestations / Invited speaker 2) Chronic Diarrhea: A Practical Approach / Invited speaker PRIMED Update in Primary Care (PRIMED) Anaheim, CA
2013	Update on Colorectal Cancer Screening Gastroenterology and Hepatology Academy Virtual Symposium
2013	Advances in Celiac Disease Diagnosis / Session Chair Digestive Diseases Week, American Gastroenterological Association San Diego, CA
2013	The True Burden of Celiac Disease: Making the Case for Prevention / Invited Speaker Digestive Diseases Week, American Gastroenterological Association San Diego, CA

2013	Celiac Disease: Presentation and Diagnosis from a Clinical and Pathologic Perspective / Invited Speaker Digestive Diseases Week, American Gastroenterological Association San Diego, CA
2013	Small Bowel Disease Board Review Session / Invited Speaker Digestive Diseases Week, American Gastroenterological Association San Diego, CA
2013	Diagnosing Celiac Disease in 2013/Invited Speaker 13th International Celiac Disease Symposium Chicago, IL
2013	Review of Gastroenterology / Course instructor ACP Review Course American College of Physicians Boston, MA
2013	1) Celiac Disease: Protean Manifestations / Invited speaker 2) Clostridium Difficile Infection / Invited speaker PRIMED Update in Primary Care (PRIMED) Boston, MA
2014	Measuring Outcomes in Celiac Disease / Invited Speaker Developing Therapies for Celiac Disease Conference New York, NY
2014	Celiac Disease/ Invited speaker PRIMED Update in Primary Care (PRIMED) Phoenix, AZ
2014	Celiac Disease/ Invited speaker PRIMED Update in Primary Care (PRIMED) Seattle, WA
2014	Celiac Disease/ Invited speaker PRIMED Update in Primary Care (PRIMED) Denver, CO
2014	Celiac Disease/ Invited speaker PRIMED Update in Primary Care (PRIMED) Houston, TX
2014	Celiac Disease/ Invited speaker PRIMED Update in Primary Care (PRIMED) Chicago, IL
2014	Small Bowel Disease Board Review Session / Invited Speaker Digestive Diseases Week, American Gastroenterological Association Chicago, IL
2014	Celiac Disease Who to Screen, How to Test and Diagnose?/Invited Speaker Digestive Diseases Week, American Gastroenterological Association Chicago, IL
2014	Update on Celiac Disease/Invited Speaker University of Colorado Medical Center Gastroenterology Grand Rounds

2014	Celiac Disease and Non Celiac Gluten Sensitivity / Invited Speaker University of Colorado Medical Center Updates in Clinical Nutrition
2015	Role of Serology to Measure Clinical Benefit and Appropriate timing of assessment in Celiac Disease / Invited Speaker FDA Gastrointestinal Regulatory Endpoints and Advancement of Therapeutics Conference on Celiac Disease
2015	Small Bowel Disease Board Review Session / Invited Speaker Digestive Diseases Week, American Gastroenterological Association Washington DC
2015	Celiac Disease Clinical Research Abstract Session/Invited Speaker Digestive Diseases Week, American Gastroenterological Association Washington DC
2015	Celiac Disease Factors Influencing Development of Disease/Session Moderator Digestive Diseases Week, American Gastroenterological Association Washington DC
2015	Celiac Disease: Protean Manifestations / Invited speaker PRIMED Update in Primary Care (PRIMED) Boston, MA
2016	Celiac Disease Grand Rounds, Norwalk Hospital, Norwalk, CT
2016	Small Bowel Disease Board Review Session / Invited Speaker Digestive Diseases Week, American Gastroenterological Association San Diego, CA
2016	New Therapeutic Approaches in Celiac Disease /Session Moderator Digestive Diseases Week, American Gastroenterological Association San Diego, CA
2016	'Help my gluten free diet isn't working!' Postgraduate course Invited Speaker Digestive Diseases Week, American Gastroenterological Association San Diego, CA
2016	New Developments in Celiac Disease Research Session/Invites Speaker Digestive Diseases Week, American Gastroenterological Association San Diego, CA
2016	Celiac Disease: Below the Tip of the Iceberg. Invited Speaker, ImmunosanT Sponsored Symposium. San Diego, CA

International *Those presentations below sponsored by outside entities are so noted and the sponsor is identified*

2007	Psychological correlates of gluten-free diet adherence / Invited speaker (Abstract) International Celiac Disease Society / 21st Annual Association of European Celiac Societies Meeting, Maribor, Slovenia
2011	The Future of Celiac Disease / Keynote address Canadian Celiac Association Annual Celiac Conference Ottawa, Canada
2011	1) Monitoring of Individuals with Celiac Disease: Knowns and Unknowns / Clinical Forum 2) Monitoring of Individuals with Celiac Disease: Knowns and Unknowns / Scientific Symposium

	International Celiac Disease Society 14th International Celiac Symposium Oslo, Norway
2012	Clinical Dilemmas in Celiac Disease / Invited speaker XII Congress of the Russian Scientific Society of Gastroenterology Moscow, Russia
2012	Celiac Disease: Modern Lessons From an Ancient Disease / Nutrition and Metabolism Workshop Palliser Primary Care Network Calgary, Ontario, Canada
2013	Celiac Disease and Cardiovascular Outcomes University of Orebro Orebro, Sweden
2014	Celiac Disease City Wide Gastroenterology Grand Rounds, University of Toronto Toronto, Canada
2014	Celiac Disease/ Plenary Session Alberta Digestive Disease Society Annual Meeting Calgary, Canada
2014	Serum Markers in Celiac Disease: Standards and Novel Approaches Dr. Falk, Small Bowel Symposium Amsterdam, Netherlands
2014	Non-Celiac Gluten Sensitivity vs. Celiac Disease: Diagnostic Approaches Third Non-Celiac Gluten Sensitivity Symposium Salerno, Italy
2015	Celiac Disease: Changes in Prevalence, Novel Diagnostics, Evaluation of Non Responsive Celiac Disease and Future Therapies Latin American Symposium on Celiac Disease/Course on Diseases of the Intestine and Colon Buenos Aires, Argentina
2016	Novel Developments in Celiac Disease Therapeutics Autoimmunity 2016 Leipzig, Germany

Report of Clinical Activities and Innovations

Current Licensure and Certification

2005	Internal Medicine License
2005	Board Certified in Internal Medicine
2008	Board Certified in Gastroenterology

Practice Activities

Ambulatory Care	Outpatient Clinic	BIDMC	One session per week
Endoscopy	Endoscopy Unit	BIDMC	One session per week
Consult Attending	Inpatient Units	BIDMC	Four weeks per year

Clinical Innovations

2008	Handheld ingredient scanner for detection of gluten containing foods. I partnered with an electronics design firm to create and market a handheld device capable of scanning food barcodes to assess for foods known or suspected to contain gluten. The aim is to decrease the burden of gluten free diet adherence and thus improve gluten avoidance, especially in those with visual or literacy deficits who are most at risk for inadvertent gluten exposure.
2010	System for Improving Endoscopy Patient Recall I led a team of gastroenterologists, primary care physicians and quality improvement specialists in a project to design, implement and study an automated system for improving patient adherence to follow up endoscopy recommendations. This work led to increased numbers of patients effectively managed for clinically significant conditions as well as peer reviewed publications and presentations at national meetings.
2012	Ex-Vivo Gluten Challenge for Diagnosis of Celiac Disease I have developed a novel method of diagnosing celiac disease in patients already on a gluten challenge using ex-vivo gluten challenge in cultured intestinal biopsies. Work is ongoing to develop and protect this innovation which has the potential to be a widely used new tool for clinical and research activities in celiac disease and other gluten related disorders.

Report of Education of Patients and Service to the Community

Activities

2006-Present	Healthy Villi Celiac Advocacy Group Presented lectures on evolving topics in celiac disease and participated in question and answer session. Quarterly with attendance of ~400 per meeting.
2007-Present	Celiac Center at BIDMC Participate in design and execution of quarterly information sessions for patients with newly diagnosed celiac disease. Attendance ~30 per meeting
2008	Celiac Center at BIDMC Participate in design and execution of a patient education forum for adults with celiac disease. Attendance ~200
2009	New England Celiac Conference Participated with the Healthy Villi in planning and delivering content at this annual regional meeting. Attendance in 2009 was over 650 individuals.
2009-2012	Celiac Center at BIDMC and at Children's Hospital, Boston Project leader for the development of an educational program for young adults with celiac disease as they plan to leave the home
2012-Present	Regular guest contributor to the patient magazine *Gluten Free Living*
2014-Present	Regular guest contributor to the patient magazine *Living Without*
2013	BIDMC Mini-Medical School Lecture: Food Related Disorders Presenter

Educational Material for Patients and the Lay Community

Patient educational material

2007	Co-Author of Celiac Disease: A Primer Patient education pamphlet

Available through the Divisions of Gastroenterology and Nutrition at BIDMC, >5,000 distributed

2010 Dennis M, **Leffler D**.
Real Life With Celiac Disease: Troubleshooting and Thriving Gluten Free
Book published by the American Gastroenterological Association
Winner of the 2011 'Indie Book Award' for Diet and Nutrition: www.indiebookawards.com
Currently sold >10,000 copies nationwide. Current rating on Amazon: 4.9 out of 5 stars

Report of Scholarship

Peer reviewed publications in print or other media

Research Investigations

1. **Leffler D.** U.S. high school age girls may be receptive to breastfeeding promotion. Journal of Human Lactation 2000;16(1):37-41

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3. **Leffler D**, Dennis M, Hyett B, Kelly E, Schuppan D, Kelly CP. Etiologies and Predictors of Diagnosis in Non-Responsive Celiac Disease. Clin Gastroenterol Hepatol. 2007 Apr;5(4):445-50

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and Hepatology. 2009 May;7(5):530-6, 536.e1-2.

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18. Kheraj R, Tewani SK, Ketwaroo G, **Leffler DA**. Quality Improvement in Gastroenterology Clinical Practice. Clin Gastroenterol Hepatol. 2012;Dec;10(12);1305-14.

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21. Mukherjee R, **Leffler DA**. Diseaeses of the Small Intestine. Digestive Diseases Self-Education Program (DDSEP) 7. American Gastroenterology Assocation. 2013

22. Vanga R, **Leffler DA**. Gluten sensitivity: not celiac and not certain. Gastroenterology. 2013 Aug;145(2):276-9. [Editorial]

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management of celiac disease. Gastroenterol Rep (Oxf). 2015 Feb;3(1):3-11. PMID: 25326000

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32. **Leffler D.A.**, Dennis M., and Kelly C.P. Celiac disease. In D.K. Podolsky, M. Camilleri, J.G. Fitz, A.N. Kalloo, F. Shanahan, and T.C. Wang (eds) 2016, Celiac Disease Yamada's Textbook of Gastroenterology, 6th ed. Oxford: John Wiley & Sons, Ltd. pp 1264–1275.

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35. Tapper EB, **Leffler DA**. The Morbidity and Mortality Conference in Gastroenterology and Hepatology: An Important Cornerstone of Patient Safety and Optimal Care. Gastroenterology. 2015 Nov 22. PMID: 26615118

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Non-peer reviewed scientific or medical publications/materials in print or other media

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2. **Leffler D.** 'Getting Serious About Celiac Disease' Online Editorial for the American Gastroenterological Association. http://www.gastro.org/journals-publications/aga-perspectives/getting-serious-about-celiac 2010 [Invited editorial]

3. **Leffler D.** 'The Vicious Cycle of Unrecognized Celiac Disease' Editorial for Gastroenterological Association publication *AGA Perspectives.* 7(1)2011

4. Kabbani TA, **Leffler DA**. Letter: rising incidence of obesity in the coeliac population - a malady or maladaptation? Authors' reply. Aliment Pharmacol Ther (2012 Jun) 35(12):1484 [Letter to the Editor]

5. Feuerstein JD, **Leffler DA**, Cheifetz AS. How physicians interpret research funding disclosures. NEJM. 2012 Dec 13;367(24):2358-9 [Letter to the Editor]

6. Feuerstein JD, **Leffler DA**. Colonoscopy and polyp characteristics. Ann Intern Med. 2013 Jan 15;158(2):141-2 [Letter to the Editor]

7. Kelly CP, Green PH, Murray JA, Dimarino A, Colatrella A, **Leffler DA**, Alexander T, Arsenescu R, Leon F, Jiang JG, Arterburn LA, Paterson BM, Fedorak RN; for the Larazotide Acetate Celiac Disease Study Group. Commentary: larazotide acetate - an exciting new development for coeliac patients? Authors' reply. Aliment Pharmacol Ther. 2013 Feb;37(4):496-497. [Letter to the Editor]

8. Feuerstein JD, **Leffler DA**, Cheifetz AS. Letter: international IBD practice guidelines - authors' reply. Aliment Pharmacol Ther. 2013 Aug;38(3):326-7. [Letter to the Editor]

9. Feuerstein JD, **Leffler DA**, Cheifetz AS. Letter: inflammatory bowel disease guidelines and conflicts of interest - authors' reply. Aliment Pharmacol Ther. 2013 Aug;38(4):445-6. [Letter to the Editor]

10. Bukoye B, **Leffler D**. Topical anesthetic-induced methemoglobinemia and veterans affairs hospitals-reply. JAMA Intern Med. 2013 Nov 25;173(21):2013-4. [Letter to the Editor]

11. Feuerstein JD, **Leffler DA**. Acute gastrointestinal bleeding. Ann Intern Med. 2013 Dec 3;159(11):793. [Letter to the Editor]

12. Rupa Mukherjee, **Daniel A. Leffler.** Digestive Diseases Self-Education Program (DDSEP) 7th edition. Chapter on small intestinal diseases - print and online published by the American Gastroenterological Association 2013[Book Chapter]

13. Natalia E. Castillo, **Daniel A. Leffler.** The Value of BCG and TNF in Autoimmunity. Editor Denise L. Faustman. Academic Press, March 2014 [Book Chapter]

14. Rohini Vanga, **Daniel A. Leffler.** GI/Liver Secrets 5th Edition. Editor Peter R. McNally. Chapter on celiac disease and small intestinal diseases. Elsevier Press 2014 [Book Chapter]

15. **Daniel A. Leffler**, Ciaran P. Kelly. Yamada's Textbook of Gastroenterology. 6th Edition. Chapter on celiac disease. Wiley Blackwell Press. 2014 [Book Chapter]

16. Kabbani TA, Vanga RR, **Leffler DA**, Villafuerte J, Pallav K, Hansen J, Mukherjee R, Dennis M, Kelly C. Response to aziz et Al. Am J Gastroenterol. 2014 Sep;109(9):1499-500. PMID:25196881 [Letter to the Editor]

17. Feuerstein JD, **Leffler DA**, Cheifetz AS. Colonoscopy is appropriately utilized in most cases following a fair bowel prep. Am J Gastroenterol. 2014 Aug;109(8):1289. PMID: 25091247[Letter to the Editor]

Professional educational materials or reports, in print or other media

1. 2005-2008 Tutorial Cases for *Gastrointestinal Pathophysiology Course 708.0* -
Worked with Drs. Helen Shields and Antoinette Peters on development of the GI pathophysiology tutorial cases to include issues of culturally competent care for second year Harvard medical and dental students.

2. 2005 Hereditary Colorectal Cancer, A Practical Review - web content
Web based learning module of hereditary colon cancer for second year Harvard medical and dental students: http://ecommons.med.harvard.edu/VResources/getResources.aspx?a=&course_id=1003093377

3. 2006 Summary of Gastrointestinal Hormones handout
Creation of learning aid for gastrointestinal hormones as part of the GI pathophysiology course for second year Harvard medical and dental students

4.	2006	'Some thoughts on a future in Gastroenterology' handout Creation of a handout describing the training, research and employment opportunities for students interested in gastroenterology for second year Harvard medical and dental students
5.	2006	Integration of cross cultural care into problem based learning module - video With Drs. Helen Shields and Antoinette Peters, I created a video demonstration of the integration of social and cultural issues in to a GI pathophysiology tutorial session for tutorial leaders and course instructors at Harvard Medical School
6.	2006	Oral Manifestations of Gastrointestinal Disease - web content Web based review of oral manifestations of gastrointestinal diseases for second year Harvard medical and dental students [website no longer active]
7.	2009	Celiac Disease: Update on Diagnosis and Treatment - web content Online CME course through Harvard Medical School Department of Continuing Education for physicians and other health care professionals
8.	2010	Nutritional Management of Celiac Disease - online slide set Online CME course through the American Gastroenterological Association for Gastroenterologists
9.	2010	Defining Diagnosing and Managing Celiac Disease - Online slide set Online CME course through the National Foundation for Celiac Awareness for Physicians and other health care professionals

Clinical Guidelines and Reports

1.	2006	1) Celiac Disease Diagnostic Algorithm 2) Celiac Disease Management Algorithm Guidelines to standardize and improve the diagnostic approach to celiac disease BIDMC Celiac Center Clinical Guidelines
2.	2007 2007 2009	1) Celiac Disease (Written with Dr. Ciaran Kelly) 2) Chronic Diarrhea (Written with Dr. Ciaran Kelly) 3) Zinc Deficiency British Medical Journal; Point-of-Care Physicians Reference Database Web based reference database focused on disease diagnosis and management. Updated annually
3.	2014	Shields HM, Atlas JS, **Leffler D**, Percac-Lima S, Sequist T, Chung D, Lim R, Roseto J, Ryou M. Prevention and Early Detection of Colorectal Cancer, A CRICO Decision Support Tool, 2014.

Abstracts, Poster Presentations and Exhibits Presented at Professional Meetings

1. Tariq S, Pallav K, Hansen J, Schuppan D, Kelly CP, **Leffler DA.** The Clinical Utility of HLA Testing in Celiac Disease Differential Diagnosis Digestive Diseases Week 2011

2. Pallav K, Tariq S, Daniel A. **Leffler DA**, Dennis M, Hansen J, Peer A, Schuppan D, Kelly CP. Serum IgA in Celiac Disease: The Unrecognized Importance of Partial IgA Deficiency. Digestive Diseases Week 2011

3. Na X, Martin A, **Leffler DA**, Flores SL, Lorraine K, Hu M, Kelly CP. Derivation and Validation of a Clinical Prediction Tool for Severe Clostridium difficile Infection Digestive Diseases Week 2011

4. Socioeconomic Status Influences Celiac Disease Diagnosis Mehra S, **Leffler DA**, Pallav K, Tariq S, Shah S, Green PH, Hansen J, Dennis M, Kelly CP Digestive Diseases Week 2011

5. Akbari M, Shah S, Kelly CP, Bhansali A, Hansen J, Dennis M, **Leffler DA.** Factors Affecting the Treatment Burden of Celiac Disease Digestive Diseases Week 2012

6. Akbari M, Shah S, Kelly CP, Bhansali A, Hansen J, Dennis M, **Leffler DA.** Socioeconomic Risk Factors for Celiac Disease Burden and Symptoms Digestive Diseases Week 2012

7. Kabbani TA, **Leffler DA**, Pallav K, Bhansali A, Dennis M, Kelly CP. Is Celiac Disease Protective Against Non-Insulin Dependent Diabetes Mellitus? Digestive Diseases Week 2012

8. Shah S, Akbari M, Kelly CP, Bhansali A, Hansen J, Dennis M, **Leffler DA.** Celiac Disease Has Higher Treatment Burden Than Common Medical Conditions Digestive Diseases Week 2012

9. Hshieh TT, Katchar K, Perera PN, Chen X, Xu H, Herzig SJ, **Leffler DA**, Kelly CP. Role of Ethnicity and IL-8 Polymorphisms in Clostridium difficile Susceptibility Digestive Diseases Week 2012

10. Ketwaroo GA, Tewani SK, Kheraj R, Raptopoulos V, **Leffler DA**. Mesenteric CT Angiography in the Evaluation and Management of Acute Lower GI Bleeding Digestive Diseases Week 2012

Narrative Report

My master's degree in nutrition, obtained from Columbia University prior to entering medical school, provided me with a strong background in the impact of nutrition on health and research methodologies which I have leveraged in my clinical and research career in gastroenterology. During my fellowship in Gastroenterology, I co-founded the Celiac Center at BIDMC and after graduation took on the role of Director of Clinical Research. Since then I have spearheaded numerous studies evaluating clinical outcomes and investigating new potential therapies for celiac disease and developing initiatives to improve patient care. This work has helped us to become one of the largest celiac disease centers in North America. Our multidisciplinary team currently cares for over 2000 patients with celiac disease and other gluten related disorders and is growing by approximately 15% annually.

Currently, I devote 20% of my time to clinical and teaching activities, 30% to research in celiac disease, and 50% to administration duties (20% celiac center and 30% gastroenterology/internal medicine administrative

and quality improvement efforts). Because of my patient centered focus in celiac disease and my work in quality improvement, I believe my area of excellence lies in clinical expertise and innovation.

Clinical expertise and innovation

My main focus in clinical innovation relates to Patient Safety and Quality Improvement where I am active as Director of Quality Improvement for the Division of Gastroenterology, Associate Director of Research for Quality Improvement in the Department of Medicine, a member of the Medical Peer Review Committee, Interventional Procedures Committee, and the Department of Medicine QI Leadership Council; and, most recently I elected to the Patient Safety Core Faculty of the Department of Medicine. I have been able to leverage my clinical research and QI skills to design and execute multiple effective interventions which have been published in high level peer reviewed journals including Archives of Internal Medicine, Gastroenterology and Gastrointestinal Endoscopy. I am co-director of the patient safety and quality improvement curriculum across medicine sub-specialty fellowships at BIDMC and am engaged in a range of patient safety and quality improvement initiatives working closely with the Silverman Institute for Health Care Quality & Safety and the Center for Healthcare Delivery, both at BIDMC. Projects have included development of a multi-disciplinary clinical care algorithm for management of gastrointestinal hemorrhage, a system for improving adherence to follow up recommendations in gastrointestinal endoscopy, and ongoing work on peri-procedural anticoagulation management and quality improvement in procedural sedation. This work has led to regional and national recognition and I currently am a member of the CRICO-RMF task force on colorectal cancer prevention and have published invited reviews of quality improvement in leading gastroenterology journals.

Contributions to Teaching and Education

Committed to the academic mission at BIDMC, I am an active participant in medical education, teaching the second year Harvard Medical School Gastrointestinal Pathophysiology Course for the past seven years and I was also the teaching fellow in 2006 working closely with Dr. Helen Shields on all aspects of course development and implementation. My specific focus in the course was the integration of cross cultural care into the problem-based learning sessions and the development of numerous novel educational tools and initiation of popular board-style quiz sessions. Through this work I earned an Excellence in Tutoring Award from the HMS Academy Center for Teaching and Learning and a nomination for the Excellence in Mentoring Award from the HMS Office of Diversity and Community Partnership. Most recently as part of my role on the Patient Safety Core Faculty at BIDMC, along with Dr. Anjala Tess, I am leading a series of training courses on patient safety and quality improvement for all clinical fellows in Department of Medicine fellowship programs at BIDMC. I also serve as Director of the Fellowship Program of the Celiac Center where I am responsible for selection, clinical oversight and mentoring of fellows in our celiac disease fellowship.

Significant Supporting Activities: Research

My efforts in celiac disease span both clinical and translational areas involving clinical outcomes and the development of novel non-invasive tests of celiac disease activity including the creation of disease specific survey tools assessing diet adherence, symptoms, and quality of life. The survey instruments in particular are used worldwide and have been translated into a number of languages. I am currently the PI on a K23 grant

assessing non-invasive markers of celiac disease activity, with a related R03 and R01 in review. I am also a close collaborator with both Dr. Detlef Schuppan in his work elucidating basic immunologic mechanisms of celiac disease, and with the celiac group at Children's Hospital. In addition, I am currently the PI on multiple industry-sponsored studies creating and evaluating celiac disease study outcomes and potential therapies. I have served as the Celiac Center Fellowship Director since 2010 and we currently train two fellows per year in a 1-2 year research and clinical training program. Papers I have authored on celiac disease have been published in top peer reviewed journals including JAMA, Gastroenterology, Gut and the American Journal of Gastroenterology. My book on celiac disease, *'Real Life with Celiac Disease: Troubleshooting and Thriving Gluten Free'* was published by the American Gastroenterological Association Press in May 2010, won an 'Indie Book Award' in 2011 and currently is rated 4.9 out of 5 stars on Amazon. Finally, my work in celiac disease has led to both national and international recognition and I currently serve as the secretary for The North American Society for the Study of Celiac Disease (www.nassed.org), and on the international 'Oslo Group' developing consensus statements for celiac disease and related disorders.

Significant Supporting Activities: Administration

Administrative duties support the quality improvement initiatives and celiac center activities and are noted in detail above. Briefly, relating to quality improvement and patient safety, I serve on multiple department of medicine and hospital wide committees and am the Director of Quality Improvement for the Division of Gastroenterology. For the Celiac Center at BIDMC, I serve as the fellowship director as well as the Director of Clinical Research.

In conclusion, over the coming years I intend to continue to devote the majority of my time to clinical and translational research in celiac disease and patient safety and quality improvement initiatives central to the mission of BIDMC. Along with these major pursuits, I will continue to balance important efforts in patient care and medical education.

Additional Materials Reviewed/Relied Upon by Daniel Leffler, M.D.

Medwatch reports and source files for Mfr Report #s

SU-2004-002638
SU-2005-004027
SU-2006-005321
SP-2006-003299
SU-2006-005596
SU-2006-005527
SU-2006-005001
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DSU-2007-00076
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Abadie V, Jabri B. *IL-15: a central regulator of celiac disease immunopathology*. Immunol Rev 2014;260:221-34.
Abdelghany M, Gonzalez L, 3rd, Slater J, et al. *Olmesartan associated sprue-like enteropathy and colon perforation*. Case Rep Gastrointest Med 2014;2014:494098.
Abenavoli L, Delibasic M, Peta V, Turkulov V, De Lorenzo A, Medic-Stojanoska M. *Nutritional profile of adult patients with celiac disease*. Eur Rev Med Pharmacol Sci 2015;19:4285-92.
Agudo Fernandez S. [*Sprue-like enteropathy due to olmesartan: a case report*]. Gastroenterol Hepatol 2015;38:108-9.
Ballou S, Bedell A, Keefer L. *Psychosocial impact of irritable bowel syndrome: A brief review*. World J Gastrointest Pathophysiol 2015;6:120-3.

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Basson M, Mezzarobba M, Weill A, et al. *Severe intestinal malabsorption associated with olmesartan: a French nationwide observational cohort study*. Gut 2015.
Benahmed M, Meresse B, Arnulf B, et al. *Inhibition of TGF-beta signaling by IL-15: a new role for IL-15 in the loss of immune homeostasis in celiac disease*. Gastroenterology 2007;132:994-1008.
Bhat N, Anupama NK, Yelsangikar A, et al. *Olmesartan-related sprue-like enteropathy*. Indian J Gastroenterol 2014;33:564-7.
Bourke CD, Berkley JA, Prendergast AJ. *Immune Dysfunction as a Cause and Consequence of Malnutrition*. Trends Immunol 2016.
Bradford Hill A. *The Environment and Disease: Association or Causation?* Proc of the Royal Society of Med, 58 (1965), 295-300.
Bresnahan KA, Tanumihardjo SA. *Undernutrition, the acute phase response to infection, and its effects on micronutrient status indicators*. Adv Nutr 2014;5:702-11.
Campos-Ruiz A, Urtasun-Arlegui L and Lopez-Valenciano C. *Sprue-like enteropathy linked to olmesartan*. Rev Esp Enferm Dig. 2016 May;108(5):292-3.
Carneiro L, Moreira A, Pereira A, Andrade C, et al. *Olmesartan-Induced Sprue Like Enteropathy*. GE Port J Gastroenterol. 2016 Apr 30;23(2):101-5. Epub 2016 Jan 14.
Cartee A, Nadeau A, Rubio-Tapia A, Herman M, and Murray J. *Characterizing Olmesartan-Induced Enteropathy Risk Factors, Severity, and Symptom Resolution, a Follow-Up to the 2012 Case Series*. AGA Abstracts Mo1271, S603-604.
Cartee AK, Murray JA. *Sprue-like Enteropathy Associated with Olmesartan*. Current Cardiovascular Risk Reports 2014;8:1-8.
Choi EY, McKenna BJ. *Olmesartan-Associated Enteropathy: A Review of Clinical and Histologic Findings*. Arch Pathol Lab Med 2015;139:1242-7.
de Araujo AA, Borba PB, de Souza FH, et al. *In a methotrexate-induced model of intestinal mucositis, olmesartan reduced inflammation and induced enteropathy characterized by severe diarrhea, weight loss, and reduced sucrose activity*. Biol Pharm Bull 2015;38:746-52.
deFonseka A, Tuskey A and Moskaluk C. *A Case of Olmesartan Induced Enteropathy*. Inflamm Bowel Dis. 2012 Dec;18:S17.
DeGaetani M, Tennyson CA, Lebwohl B, et al. *Villous atrophy and negative celiac serology: a diagnostic and therapeutic dilemma*. Am J Gastroenterol 2013;108:647-53.
Desruisseaux C, Bensoussan M, Desilets E, et al. *Adding Water to the Mill: Olmesartan-Induced Collagenous Sprue-A Case Report and Brief Literature Review*. Can J Gastroenterol Hepatol 2016;2016:4837270.
Dreifuss SE, Tomizawa Y, Farber NJ, et al. *Spruelike enteropathy associated with olmesartan: an unusual case of severe diarrhea*. Case Rep Gastrointest Med 2013;2013:618071.
Elfstrom P, Granath F, Ekstrom Smedby K, et al. *Risk of Lymphoproliferative Malignancy in Relation to Small Intestinal Histopathology Among Patients with Celiac Disease*. J Natl Cancer Inst 2011; 103:436-444.
El-Salhy M. *Irritable bowel syndrome: diagnosis and pathogenesis*. World J Gastroenterol 2012;18:5151-63.
Esteve M, Temino R, Carrasco A, et al. *Potential coeliac disease markers and autoimmunity in olmesartan induced enteropathy: A population-based study*. Dig Liver Dis 2016;48:154-61.
Eusebio M, Caldeira P, Antunes A Ramos A, et al. *Olmesartan-Induced Enteropathy: An Unusual Cause of Villous Atrophy*. GE Port J Gastroenterol. 2016 Apr 30;23(2):91-5. Epub 2015 Oct 20.

Additional Materials Reviewed/Relied Upon by Daniel Leffler, M.D.

Famularo G, Minisola G. *Relapsing Olmesartan-Associated Ileitis*. Ann Pharmacother 2016.
Fiorucci G, Puxeddu E, Colella R, et al. *Severe spruelike enteropathy due to olmesartan*. Rev Esp Enferm Dig 2014;106:142-4.
Fukushima M, Kitamoto H, Inokuma T, et al. *Severe spruelike enteropathy associated with olmesartan observed by double-balloon enteroscopy*. Gastrointest Endosc 2016;83:269-70.
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Gaur V, Albeldawi M and Weber L. *Chronic Diarrhea and Weight Loss*. Gastroenterology. 2014 Feb;146(2):347-591. Epub 2013 Dec 14.
Grath AM, Lowerison J and MacKay E. *Persistent international normalized ratio (INR)elevation associated with olmesartan-induced enteropathy in a patient receiving warfarin*. JTH 2015;13(2):755.
Greywoode R, Braunstein ED, Arguelles-Grande C, et al. *Olmesartan, other antihypertensives, and chronic diarrhea among patients undergoing endoscopic procedures: a case-control study*. Mayo Clin Proc 2014;89:1239-43.
Halevy D, Teeuwen U, Kohlhof P. [*A new spruelike disease as a cause of severe diarrhea*]. Dtsch Med Wochenschr 2014;139:2290-3.
Hallert C, Grant C, Grehn S, et al. *Evidence of poor vitamin status in coeliac patients on a gluten-free diet for 10 years*. Aliment Pharmacol Ther 2002;16:1333-9.
Hammoudi N, Dior M, Giraud V and Coffin B. *Olmesartan-induced enteropathy associated with cutaneous lesions*. Clin Case Rep. 2016 Mar 2;4(4):379-82.
Hartranft ME, Regal RE. *"Triple Phase" Budesonide Capsules for the Treatment of Olmesartan-Induced Enteropathy*. Ann Pharmacother 2014;48:1234-1237.
Hauser W, Gold J, Stein J, Caspary WF, Stallmach A. *Health-related quality of life in adult coeliac disease in Germany: results of a national survey*. Eur J Gastroenterol Hepatol 2006;18:747-54.
Hauser W, Janke KH, Klump B, Gregor M, Hinz A. *Anxiety and depression in adult patients with celiac disease on a gluten-free diet*. World J Gastroenterol 2010;16:2780-7.
Heerasing N, Hair C, Wallace S. *Olmesartan-induced enteropathy*. Intern Med J 2015;45:117-8.
Herman ML, Rubio-Tapia A, Wu TT, et al. *A Case of Severe Sprue-Like Enteropathy Associated With Valsartan*. ACG Case Rep J 2015;2:92-4.
http://www.fda.gov/Drugs/DrugSafety/ucm359477.htm.
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11/08/2010 Analysis of Reactions Related to Diarrhea and Oedema Peripheral for Olmesartan medoxomil and its combinational products

01/14/2010 Celiac Disease and Olmesartan Medoxomil – An Analysis of the Daiichi Sankyo Global Safety Database

09/28/2012 Olmesartan and Sprue-like Enteropathy Report

Exhibit J

ORIGINAL ARTICLE

Severe intestinal malabsorption associated with olmesartan: a French nationwide observational cohort study

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ABSTRACT

Objectives Severe sprue-like enteropathy associated with olmesartan has been reported, but there has been no demonstration of an increased risk by epidemiological studies.

Aim To assess, in a nationwide patient cohort, the risk of hospitalisation for intestinal malabsorption associated with olmesartan compared with other angiotensin receptor blockers (ARB) and ACE inhibitors (ACEIs).

Design From the French National Health Insurance claim database, all adult patients initiating ARB or ACEI between 1 January 2007 and 31 December 2012 with no prior hospitalisation for intestinal malabsorption, no serology testing for coeliac disease and no prescription for a gluten-free diet product were included. Incidence of hospitalisation with a discharge diagnosis of intestinal malabsorption was the primary endpoint.

Results 4 546 680 patients (9 010 303 person-years) were included, and 218 events observed. Compared with ACEI, the adjusted rate ratio of hospitalisation with a discharge diagnosis of intestinal malabsorption was 2.49 (95% CI 1.73 to 3.57, p<0.0001) in olmesartan users. This adjusted rate ratio was 0.76 (95% CI 0.39 to 1.49, p=0.43) for treatment duration shorter than 1 year, 3.66 (95% CI 1.84 to 7.29, p<0.001) between 1 and 2 years and 10.65 (95% CI 5.05 to 22.46, p<0.0001) beyond 2 years of exposure. Median length of hospital stay for intestinal malabsorption was longer in the olmesartan group than in the other groups (p=0.02). Compared with ACEI, the adjusted rate ratio of hospitalisation for coeliac disease was 4.39 (95% CI 2.77 to 6.96, p<0.0001) in olmesartan users and increased with treatment duration.

Conclusions Olmesartan is associated with an increased risk of hospitalisation for intestinal malabsorption and coeliac disease.

Significance of this study

What is already known on this subject?
- Cases of olmesartan-induced severe sprue-like enteropathy have been reported.
- The reality of the association has been questioned.
- It is also unknown whether there is an association between enteropathy and other angiotensin receptor blockers (ARBs).

What are the new findings?
- In this large nationwide observational patient cohort, olmesartan exposure is associated with an increased risk of hospitalisation for intestinal malabsorption and coeliac disease.
- This relative risk increases with treatment duration.
- We found no such risk for other ARBs.

How might it impact on clinical practice in the foreseeable future?
- Patients and physicians, including gastroenterologists, should be widely informed of this severe complication.

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INTRODUCTION

Olmesartan is an angiotensin II receptor blocker (ARB); its prodrug, olmesartan medoxomil, has been first approved in 2002 in the USA and in 2003 in the European Union, for the treatment of hypertension. Severe sprue-like enteropathies associated with olmesartan have recently been reported.[1 2] The first case series included 22 patients. These patients had severe, chronic diarrhoea and weight loss. Duodenal biopsies showed villous atrophy and inflammation. Coeliac disease serology was negative, and gluten-free diet was ineffective. All patients had taken olmesartan for several months or years. Olmesartan withdrawal was followed by clinical and, when assessed, histological improvement. Nine additional case reports and one literature review have been published[3–8] and confirmed these findings. Olmesartan seems to account for a significant proportion of non-coeliac sprues. In a series of 72 adult patients with villous atrophy and negative coeliac disease serology, olmesartan was prescribed in 16 of these patients, and all but one obtained clinical improvement after olmesartan discontinuation.[9] More recently, a new series of 39 patients with olmesartan-associated sprue has been reported.[10] Interruptions and reintroductions could be studied in a subgroup of 12 patients. Interruptions were followed by remissions, and reintroductions were followed by relapses. These reports suggest that olmesartan may cause severe enteropathy. However, the level of evidence of case reports and small series is limited. The association between olmesartan and enteropathy has also been questioned, as the ROADMAP trial, a

large randomised controlled trial with several years of follow-up, did not demonstrate any difference in diarrhoea or GI event rates between olmesartan and placebo.[11–13] However, in 2011, the FDA requested a Mini-Sentinel modular programme report of risk assessment because the number of cases of coeliac disease among users of olmesartan was higher than expected in the FDA Adverse Event Report System. The incidence of coeliac disease was found to be similar among all ARBs, including olmesartan.[14 15] Nevertheless, in July 2013, the FDA issued a 'Drug Safety Communication' approving a label change to include sprue-like enteropathy linked to olmesartan.

The association between olmesartan and enteropathy needs to be further investigated. The causality of the association remains uncertain, and its magnitude has not been determined. Moreover, it is unknown whether the association between enteropathy and ARB is limited to olmesartan or also includes other ARBs.

The objective of this study was to assess the risk of enteropathy associated with olmesartan. However, no specific diagnosis code is available for this disease, which was unknown prior to the first published case series. We, therefore, assessed the risk of intestinal malabsorption and coeliac disease associated with the prescription of olmesartan. For this purpose, we compared the rates of hospitalisation with a discharge diagnosis of intestinal malabsorption in patients who were prescribed olmesartan, other ARBs and ACE inhibitor (ACEI) in a large nationwide patient cohort.

METHODS

Data sources

The SNIIRAM (*Système National d'Information Interrégimes de l'Assurance Maladie*) is the French National Health Insurance anonymised claim database. Claims from the general health insurance scheme were used in this study. They include 51.3 million of the 65.7 million inhabitants of France (2013 census), and are available since 2006. Anonymised patient-level records contain billable claims and sociodemographic data such as age and sex. Billable claims include dispensed drugs, laboratory tests (without their results), medical procedures and ambulatory medical care. This database has been previously described.[16 17]

The French hospital discharge database programme médicalisé des systèmes d'information (PMSI) contains information about each patient admitted to a public or private hospital in France, including inpatients and outpatients. This database contains information such as discharge diagnosis (recorded by International Classification of Diseases 10 (ICD-10) code), comorbidities, age, sex, diagnosis-related group, medical procedure performed and length of stay.[16–18]

These two databases were linked in the present study in order to correlate drug prescription with hospitalisation rates and diagnoses. This study was approved by the French data protection agency (*Commission Nationale de l'Informatique et des Libertés*). All databases used in this study only contained anonymous patient records.

Study population

A cohort was constructed from the SNIIRAM, including all adult patients who initiated treatment with an ACEI or ARB between 1 January 2007 and 31 December 2012. The first filled prescription of ACEI or ARB during this period of time constituted the entry date in the cohort (index date). Patients had to be enrolled in the database for at least 1 year before the index date to prevent left censoring. To ensure the absence of left censoring, patients were required to have at least one recorded claim of any type, 1–2 years before the index date. In order to limit the study to incident users of studied drugs, we excluded patients who had filled a prescription containing ACEI or ARB during the 12 months before the index date. Patients with at least one of the following criteria were also excluded: (1) hospitalisation with a discharge diagnosis of intestinal malabsorption (ICD-10 codes K90x) during the year before the index date, (2) any filled prescription containing a gluten-free diet product during the year before the index date, (3) any reimbursed coeliac disease-specific serological testing during the year before the index date.

The ICD codes of coeliac disease and malabsorption were considered as proxies for the diagnosis of olmesartan-associated sprue. It was, therefore, necessary to exclude patients with history of intestinal malabsorption before index date and/or patients with coeliac disease. Therefore, patients who had undergone serological testing or had received gluten-free diet or had been hospitalised with a discharge diagnosis of intestinal malabsorption before the index date were excluded.

Outcomes

The primary outcome was hospitalisation with a discharge diagnosis of intestinal malabsorption (ICD-10 codes K90x). The secondary outcome was hospitalisation with a discharge diagnosis of coeliac disease (ICD-10 code K90.0). Patients were censored at the first event, death or end of the study (31 December 2012 in the main analysis and 31 May 2012 in the sensitivity analysis to avoid information bias).

Exposure assessment

Three kinds of exposures were studied: exposure to olmesartan, exposure to other ARB and exposure to ACEI. Exposure was defined as follows for these three groups. It started from the date of a filled prescription containing a drug of interest (ie, olmesartan, other ARB or ACEI). The end of exposure was defined as the end of prescription duration plus a grace period of 30 days. Grace period is commonly used and recommended in pharmacoepidemiological studies based on claim databases in order to account for incomplete medication adherence and avoid underestimation of drug exposure or misattribution of events. Patient could simultaneously fall into several exposure categories (eg, ACEI+olmesartan). Such periods of overlapping exposure to different drug class were removed from the analysis to prevent misattribution of events. However, they were accounted for in the calculation of treatment duration to prevent classification bias.

Statistical methods

For the primary outcome, a Poisson regression model adjusted for the following potential confounders was used: age, sex, heart failure, dementia, diabetes, immune-mediated diseases (rheumatoid arthritis, Hashimoto thyroiditis, IgA deficiency, dermatitis herpetiformis, lupus, Sjogren, dermatopolymyositis, complement deficiency, angioedema, IBDs), transplantation, ongoing cancer and renal failure. The comorbidities were based on the diagnoses, medical procedures and drug prescriptions from the PMSI and the SNIIRAM.

For the secondary outcome (hospitalisation with a discharge diagnose of coeliac disease), the Poisson regression model was adjusted for age, sex and the following comorbidities: heart failure, diabetes, immune-mediated diseases (rheumatoid arthritis, Hashimoto thyroiditis, IgA deficiency, dermatitis herpetiformis, lupus, Sjogren, dermatopolymyositis, complement deficiency, angioedema, IBDs), active cancer and renal failure.

Dementia and transplantation were removed because of a lack of events. We adjusted for these comorbidities for the following reasons. Patients with immune-mediated abnormalities are at increased risk for coeliac disease. Patients with cancer or allograft recipients are often prescribed drugs that may provoke diarrhoea and malabsorption. Patients with dementia are commonly treated differently from other patients regardless of the disease. Diabetes is a common cause of GI symptoms, including diarrhoea (autonomous neuropathy). Renal failure and heart failure may have influenced the choice of antihypertensive drug.

Poisson regression model fit was assessed by overdispersion analysis, using the deviance/number of degree of freedom ratio and the Pearson χ^2 statistic. Medians were compared by the multisample median test (Brown–Mood test), which assigns 1 for observations greater than the median, and 0 otherwise, and produces χ^2 statistics.[19] Data management and statistical analyses were performed with SAS Enterprise Guide V4.3.

RESULTS

Study population

A total of 4 552 130 patients initiating ARB or ACEI treatment between 2007 and 2012 were selected from the database; 154 patients who had been hospitalised for intestinal malabsorption during the 12 months preceding inclusion and 4611 patients who had undergone coeliac disease serology testing during the past 12 months were excluded. Finally, 685 patients with a reimbursement claim for a gluten-free diet product in the past 12 months were also excluded. A total of 4 546 680 patients corresponding to 9 129 149 person-years (PY) were included: 118 846 PY of multiple exposures were excluded from the analysis and the remaining 9 010 303 PY of single treatment exposure were distributed as follows: 3 646 311 PY of ACEI exposure, 860 894 PY of olmesartan exposure and 4 503 098 PY of other ARB exposure. The inclusion flow chart is presented in figure 1.

Baseline patient characteristics are presented in table 1. Mean age at inclusion was 63.9 years in the ACEI group, 61.3 years in the olmesartan group and 62.3 years in the other ARB group. The ACEI group comprised fewer women (45.6%) than the olmesartan group (53.9%) and the other ARB group (55.6%). The Poisson regression model was adjusted for both age and sex.

Seventy-seven per cent of the PY in the olmesartan group were included during the 2010–2012 period compared with 72% in the ACEI group and 70% in the other ARB group. Median duration of treatment exposure varied from 326 days (ACEI) to 348 days (olmesartan) and 514 days (other ARBs).

No coding trend of hospital discharge diagnoses of intestinal malabsorption was observed over the study period (see online supplementary table S2).

Incidence of severe malabsorption and coeliac disease

Two hundred eighteen hospitalisations for intestinal malabsorption were observed, 87 in the ACEI group, 48 in the olmesartan group and 83 in the other ARB group, yielding crude incidence rate of 2.4 per 100 000 PY, 5.6 per 100 000 PY and 1.8 per 100 000 PY, respectively.

Olmesartan was associated with an adjusted rate ratio of 2.49 (95% CI 1.73 to 3.57, p<0.0001) of hospitalisation with a discharge diagnosis of intestinal malabsorption compared with ACEI and a rate ratio of 3.17 (95% CI 2.22 to 4.53, p<0.0001) compared with other ARBs. ARBs other than olmesartan were associated with a non-significant rate ratio of 0.78 (95% CI 0.58 to 1.07, p=0.12) of hospitalisation with a discharge diagnosis of intestinal malabsorption, compared with ACEI. Women had a higher rate ratio of hospitalisation with a discharge diagnosis of intestinal malabsorption (rate ratio 1.42, 95% CI 1.08 to 1.87, p=0.01). Inclusion of an interaction term between sex and treatment was added to the model, but was not significant, and was, therefore, not kept in the multivariate model. Gender-stratified results were also calculated. We found no difference between men and women (data not shown). Age had no influence on this rate ratio.

Median length of hospital stay was longer in the olmesartan group (9 days) than in the other ARB group (2 days) and the ACEI group (4 days) (p=0.02).

Hospitalisations with a discharge diagnosis of coeliac disease (ICD-10 code K90.0) were also studied, as olmesartan-associated enteropathy mimics coeliac disease. Adjusted rate ratio of hospitalisation with a discharge diagnosis of coeliac disease was 4.39 (95% CI 2.77 to 6.96, p<0.0001) in patients who were prescribed olmesartan compared with those who were

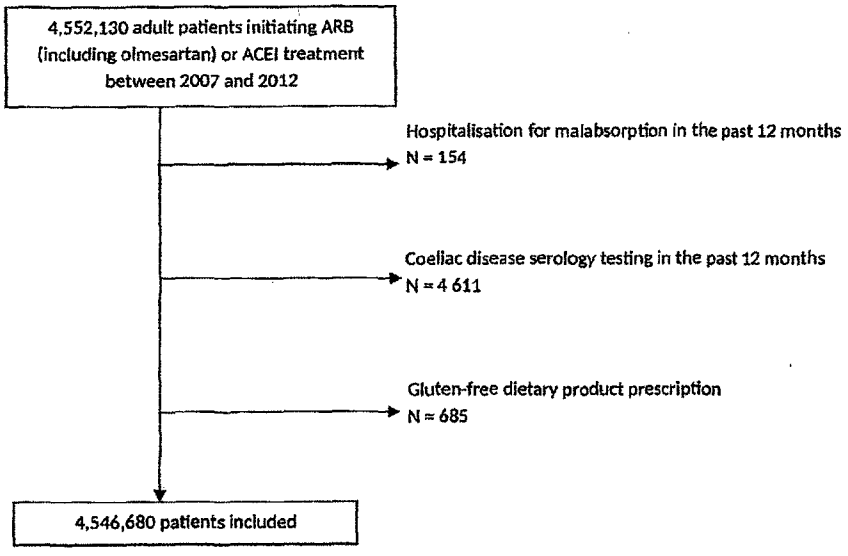

Figure 1 Inclusion flow chart. ACEI, ACE inhibitor; ARB, angiotensin receptor blocker.

Table 1 Population characteristics

	ACEI			Other ARBs			Olmesartan		
	Number of PY	Per cent	Number of events	Number of PY	Per cent	Number of events	Number of PY	Per cent	Number of events
Total	3 646 311	100	87	4 503 098	100	83	860 894	100	48
Women	1 662 055	45.6	40	2 504 538	55.6	59	464 166	53.9	31
Age									
18–39 years	117 367	3.2	8	145 315	3.2	2	30 515	3.5	1
40–49 years	373 726	10.2	8	495 596	11.0	13	108 604	12.6	6
50–59 years	822 079	22.5	22	1 082 446	24.0	13	227 431	26.4	7
60–69 years	921 633	25.3	25	1 195 828	26.6	19	235 147	27.3	12
70–79 years	788 347	21.6	14	975 638	21.7	24	172 525	20.0	14
≥80 years	623 159	17.1	10	608 274	13.5	12	86 672	10.1	8
Inclusion year									
2007	123 472	3.4	3	165 809	3.7	4	22 987	2.7	1
2008	337 993	9.3	17	461 097	10.2	9	66 881	7.8	1
2009	544 289	14.9	12	706 453	15.7	18	111 884	13.0	3
2010	729 240	20.0	23	906 922	20.1	21	161 780	18.8	10
2011	878 875	24.1	13	1 067 364	23.7	13	216 958	25.2	18
2012	1 032 443	28.3	19	1 195 453	26.5	18	280 405	32.6	15

ACEI, ACE inhibitor; ARB, angiotensin receptor blocker; PY, person-years.

prescribed ACEI and 4.82 (95% CI 3.12 to 7.45, p<0.0001) compared with other ARBs. This ratio was 0.91 (95% CI 0.58 to 1.42, p=0.68) in patients who were prescribed other ARBs compared with those who were prescribed ACEI. See table 4 for details.

The first case report linking olmesartan and enteropathy was published online on 25 June 2012. We, therefore, performed a sensitivity analysis in which the study period and follow-up ended on 31 May 2012, which gave very similar results (see online supplementary tables S3–S5).

Risk over time

Descriptive data were in favour of non-homogeneity of risk according to the duration of treatment exposure (table 2). To account for such changes in risk and to assess the kinetics of the risk of hospitalisation with a discharge diagnosis of intestinal malabsorption associated with olmesartan exposure, the model was stratified on treatment exposure. The following duration strata were used: less than 1 year, between 1 and 2 years, and 2 years or more. Compared with ACEI, the adjusted rate ratio of hospitalisation with a discharge diagnosis of intestinal malabsorption associated with olmesartan exposure was 0.76 (95% CI 0.39 to 1.49, p=0.43) for treatment duration shorter than 1 year, 3.66 (95% CI 1.84 to 7.29, p<0.001) between 1 and 2 years of treatment exposure and 10.65 (95% CI 5.05 to 22.46, p<0.0001) beyond 2 years of treatment exposure (table 3). Very similar results were obtained when follow-up ended on 31 May 2012 (see online supplementary tables S4 and S5). Compared with ACEI, the rate ratio of hospitalisation with a discharge diagnosis of coeliac disease was 1.98 (95% CI 0.85 to 4.61, p=0.11) for treatment shorter than 1 year; 4.36 (95% CI 2.04 to 9.34, p<0.001) for treatment between 1 and 2 years and 10.21 (95% CI 4.21 to 24.76, p<0.0001) for more than 2 years of olmesartan exposure (table 4). Details of discharge diagnoses by duration of treatment exposure in each group are presented in online supplementary table S1. No overdispersion was observed in any Poisson regression models.

Table 2 Risk over time: descriptive data

	ACEI	Olmesartan	ARB
PY	3 646 311	860 894	4 503 098
0–1 year	1 584 921	377 748	1 706 722
1–2 years	922 124	223 477	1 153 054
≥2 years	1 139 266	259 668	1 643 322
Number of events	87	48	83
0–1 year	59	10	36
1–2 years	18	15	23
≥2 years	10	23	24
Crude incidence rate (per 100 000 PY)	2.39	5.58	1.84
0–1 year	3.72	2.65	2.11
1–2 years	1.95	6.71	1.99
≥2 years	0.88	8.86	1.46

ACEI, ACE inhibitor; ARB, angiotensin receptor blocker; PY, person-years.

DISCUSSION

In this large nationwide cohort of patients, olmesartan users were found to have an increased risk of hospitalisation for intestinal malabsorption and coeliac disease compared with ACEI. These risks increased with duration of olmesartan exposure up to 10-fold beyond 2 years of exposure. Users of ARBs other than olmesartan did not exhibit an increased risk of hospitalisation for intestinal malabsorption or coeliac disease. These results were adjusted for potential confounders. During the first year of treatment, patients treated with other ARBs had a decreased rate of hospitalisation for intestinal malabsorption compared with patients treated with ACEI. There was an excess of diagnoses of malabsorption other than coeliac disease among ACEI users (ICD-10 codes K90.4, K90.8 and K90.9; see online supplementary table S1). However, no significant difference in terms of risk of hospitalisation for coeliac disease (ICD-10 code K90.0) was observed between users of ARBs other than olmesartan and ACEI users. The reason for this is unclear, but it does not affect the consistency of the results. It may have

Table 3 Crude and adjusted rate ratios of hospitalisation with a discharge diagnosis of intestinal malabsorption over time (ref: ACEI)

	Crude rate ratio	95% CI	p Value	Adjusted rate ratio	95% CI	p Value
Overall population						
Olmesartan	2.34	(1.64 to 3.32)	<0.0001	2.49	(1.73 to 3.57)	<0.0001
Other ARBs	0.77	(0.57 to 1.04)	0.09	0.78	(0.58 to 1.07)	0.12
Treatment duration <1 year						
Olmesartan	0.71	(0.36 to 1.39)	0.32	0.76	(0.39 to 1.49)	0.43
Other ARBs	0.57	(0.37 to 0.86)	0.007	0.58	(0.38 to 0.88)	0.01
Treatment duration 1–2 years						
Olmesartan	3.44	(1.73 to 6.82)	0.0004	3.66	(1.84 to 7.29)	<0.001
Other ARBs	1.02	(0.55 to 1.89)	0.95	1.03	(0.56 to 1.92)	0.92
Treatment duration >2 years						
Olmesartan	10.09	(4.80 to 21.20)	<0.0001	10.65	(5.05 to 22.46)	<0.0001
Other ARBs	1.66	(0.80 to 3.48)	0.18	1.68	(0.80 to 3.51)	0.18

ACEI, ACE inhibitor; ARB, angiotensin receptor blocker.

underestimated the rate ratio associated with olmesartan as compared with ACEI.

The strength of the association and the consistency with reported cases (including the long lag time between initiation of olmesartan and diagnosis of malabsorption) are strong arguments in favour of causality. In addition, the longer length of hospital stay in patients who were prescribed olmesartan suggests that their disease was distinct from and more severe than that observed in patients receiving ARBs or ACEI. Patients who obtained clinical improvement after stopping olmesartan and who experienced subsequent recurrence of symptoms on olmesartan rechallenge have also been described.[6 7] In the aforementioned ROADMAP trial, no significant difference in the rate of GI adverse events or diarrhoea was observed between olmesartan and placebo.[10–12] However, these adverse events are common in patients with diabetes (reported in 3.5% and 2.3% of patients in the olmesartan arm of this trial, respectively), and may have confounded the effect of olmesartan on the risk of severe enteropathy. This more specific risk was not assessed in this trial, which did not have sufficient statistical power to detect such an association. For the same reasons, a recent cohort study did not find any significant difference in the risk of GI disease-related hospitalisation among patients with diabetes treated by olmesartan compared with patients with diabetes treated by other ARBs.[20]

Table 4 Adjusted rate ratios of hospitalisation with a discharge diagnosis of coeliac disease (ref: ACEI)

	Adjusted rate ratio	95% CI	p Value
Overall population			
Olmesartan	4.39	(2.77 to 6.96)	<0.0001
Other ARBs	0.91	(0.58 to 1.42)	0.68
Treatment duration <1 year			
Olmesartan	1.98	(0.85 to 4.61)	0.11
Other ARBs	1.07	(0.56 to 2.05)	0.84
Treatment duration 1–2 years			
Olmesartan	4.36	(2.04 to 9.34)	<0.001
Other ARBs	0.77	(0.36 to 1.67)	0.51
Treatment duration >2 years			
Olmesartan	10.21	(4.21 to 24.76)	<0.0001
Other ARBs	0.94	(0.36 to 2.47)	0.90

ACEI, ACE inhibitor; ARB, angiotensin receptor blocker.

This study has several strengths. First, it was based on a large nationwide database. Second, we adjusted for potential confounders that may affect the outcome (hospitalisation with a discharge diagnosis of malabsorption) or the prescription of antihypertensive drugs. Finally, to prevent selection bias, we excluded those patients with malabsorption and those at risk for coeliac disease before the index date.

Several potential limitations of this study should also be discussed. First, this study was based on administrative data, which may result in information bias. There is no direct comparison between these data and chart review in France for the diagnosis of intestinal malabsorption or coeliac disease. However, the possible lack of sensitivity is unlikely to affect the three groups of the study differently; as such, it does not result in bias in the analysis, and could not refute the message of the study. Another issue raised by healthcare electronic records concerns trends in coding practice. However, in this study, no coding trend was observed for intestinal malabsorption among adult patients in France during the study period (see online supplementary table S2). Second, the potential indication bias should be discussed. However, ACEI and ARB share very similar therapeutic indications. Coeliac disease is more frequent in women and in younger subjects,[21] but analyses were adjusted for age and sex. In addition, there is no reason why coeliac disease-predisposing HLA genotype would be overrepresented in patients who were prescribed olmesartan. Finally, it is unlikely that all cases of olmesartan-associated enteropathy were captured by hospital diagnoses of intestinal malabsorption and coeliac disease. It is likely that milder forms also exist. Overall number needed to harm was 31 350 patient-years of olmesartan exposure. Beyond 2 years of exposure, this number was 12 500 patient-years. However, caution is needed to interpret these values as this study was not aimed to measure the incidence of olmesartan-associated enteropathy, but rather to estimate the strength of the association between olmesartan and severe forms of enteropathy and malabsorption. As a consequence, this study underestimates the true incidence and only provides the incidence of the most severe forms of olmesartan-associated enteropathy.

In summary, this paper shows, with a higher level of evidence, the association between severe intestinal malabsorption and olmesartan exposure. These results have important practical consequences as olmesartan is widely prescribed worldwide. In France, olmesartan was prescribed to more than 800 000 patients in 2012. Patients treated with olmesartan should be informed about the risk of this complication, and should be

advised to seek medical attention if they experience GI symptoms. This information should also be widely delivered to physicians of all disciplines, particularly to gastroenterologists who are faced to this new category of patients.

However, further studies are required to assess the frequency and clinical spectrum of milder forms. The pathophysiology of olmesartan-associated enteropathy also requires further investigation: the clinical and pathological features are remarkably similar to those of coeliac disease or refractory sprue, but the underlying cause and mechanisms are different. We expect such studies to shed new light on coeliac disease.

Contributors FC and HA had the idea for the study. MB conceived and planned the study and drafted the manuscript. MM performed data management and statistical analyses. All authors contributed to interpretation of the data and revised the manuscript. All authors approved the final manuscript.

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Competing interests None declared.

Ethics approval This research was authorised by the French Data Protection Agency (CNIL).

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement FC had full access to all of the data in the study, and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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GUT

Severe intestinal malabsorption associated with olmesartan: a French nationwide observational cohort study

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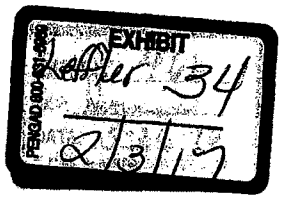
Gut published online August 6, 2015

Exhibit K



Olmesartan and Intestinal Adverse Effects in the ROADMAP Study

To the Editor: We read the article by Rubio-Tapia and colleagues[1] with great interest. In this article, the authors describe the occurrence of severe spruelike enteropathy in 22 patients, all of whom received olmesartan (predominantly 40 mg/d) besides other drugs. All patients had long-lasting diarrhea (3-53 months) and weight loss (2.5-50 kg). Many patients also experienced nausea and vomiting (68% of patients), abdominal pain (50%), bloating (41%), and fatigue (68%). Interestingly, these symptoms disappeared after use of olmesartan was stopped. The authors draw the conclusion that olmesartan may directly be involved in spruelike enteropathy. However, our observation in a large group of diabetic patients treated with 40 mg of olmesartan daily does not support this conclusion. We detected no association between treatment with 40 mg of olmesartan once daily and the occurrence of intestinal adverse effects in 2232 patients treated for a median of 3.2 years in the Randomised Olmesartan and Diabetes Microalbuminuria Prevention (ROADMAP) study.

The largest prospective, randomized, double-blind study with olmesartan is the ROADMAP study.[2] In this study, patients with type 2 diabetes were treated with 40 mg of olmesartan (n=2232) or placebo (n=2215) once daily for a median of 3.2 years, and the occurrence of microalbuminuria (interpreted as an early sign of kidney and vascular damage) was the primary end point. We now analyzed the treatment-emergent adverse events (TEAEs) reported by the study physicians. For this analysis, we selected all intestinal illnesses that typically present with diarrhea and selected all symptoms and conditions that were related to abdominal discomfort, such as pain (Table). A total of 78 patients (3.5%) in the olmesartan arm and 94 (4.2%) in the placebo arm had at least 1 episode of diarrhea or diarrhea-associated diseases. We also observed no difference between the groups in the occurrence of any intestinal TEAE. The incidence of abdominal pain or related symptoms was also comparable (Table). In the olmesartan group, 127 patients (5.7%) experienced at least 1 episode of abdominal discomfort vs 125 (5.6%) in the placebo group. The reported incidences of fatigue and weight decrease were also similar. Furthermore, we determined whether more patients prematurely terminated study participation because of intestinal or abdominal discomfort–related TEAEs. Three patients in the olmesartan group (all 3 having diarrhea) and 3 patients in the placebo group (2 having diarrhea and 1 having gastroenteritis) stopped taking the study medication because of specific gastrointestinal findings. Eight additional patients in each of the 2 study arms stopped taking the study medication because of abdominal discomfort–associated TEAEs not specifically linked to the gastrointestinal tract.

TABLE. Gastrointestinal TEAEs Reported in the ROADMAP Database

Event	No. (%) of patients: Olmesartan, 40 mg (n=2232)	Placebo (n=2215)	*P* value
Intestinal-associated TEAE	78 (3.5)	94 (4.2)	.20
Diarrhea	51 (2.3)	52 (2.3)	
Gastroenteritis	17 (0.8)	25 (1.1)	
Colitis	1	6 (0.3)	
Enteritis	2 (0.1)	4 (0.2)	
Gastroduodenitis	4 (0.1)	2 (0.1)	
Colitis, ulcerative	2 (0.1)	2 (0.1)	
Duodenitis	2 (0.1)	2 (0.1)	
Gastrointestinal disorder	3 (0.1)	1	
Gastrointestinal infection	1	3 (0.1)	
Enteritis, infectious	0	2 (0.1)	
Abdominal discomfort–associated TEAE	127 (5.7)	125 (5.6)	.95
Abdominal pain	61 (2.7)	52 (2.3)	
Upper	26 (1.2)	24 (1.1)	
Lower	2 (0.1)	1	
Location not reported by physician	33 (1.4)	27 (1.2)	
Dyspepsia	34 (1.5)	29 (1.3)	
Nausea	30 (1.3)	34 (1.5)	
Vomiting	13 (0.6)	13 (0.6)	
Flatulence	6 (0.3)	9 (0.4)	
Abdominal discomfort	4 (0.2)	4 (0.2)	
Irritable bowel syndrome	2 (0.1)	3 (0.1)	
Epigastric discomfort	2 (0.1)	2 (0.1)	
Gastrointestinal pain	1	0	
Fatigue	25 (1.1)	20 (0.9)	
Weight decrease	17 (0.8)	11 (0.5)	

ROADMAP = Randomised Olmesartan and Diabetes Microalbuminuria Prevention; TEAE = treatment-emergent adverse event.

In summary, in more than 2200 patients taking high-dose olmesartan for more than 3 years, we did not observe an intestinal effect of olmesartan. In the ROADMAP study, we could not find a link between the occurrence of diarrhea-associated complications and the intake of 40 mg/d of olmesartan. This finding might be because spruelike enteropathy is a rare event. Indeed, the 22 reported cases in the report by Rubio-Tapia et al came from 16 different states and were diagnosed at the Mayo Clinic during a time frame of 3 years. We cannot rule out the possibility

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that in this very rare disease the intestinal renin-angiotensin system plays a role; however, our data from the ROADMAP database did not identify a link between olmesartan use and the occurrence of gastrointestinal disease.

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Potential Competing Interests: Both authors have received honoraria for lectures from Daiichi-Sankyo. Dr Haller is a medical advisor to Daiichi-Sankyo and was supported by research grants.

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http://dx.doi.org/10.1016/j.mayocp.2012.10.005

Small Bowel Histopathologic Findings Suggestive of Celiac Disease in an Asymptomatic Patient Receiving Olmesartan

To the Editor: Rubio-Tapia et al[1] recently reported a possible association of olmesartan therapy with an unexplained severe enteropathy symptomatically resembling celiac disease (CD) or sprue. The 22 patients described were seen at Mayo Clinic in the relatively short period of August 1, 2008, to August 1, 2011. The usual presentation was chronic diarrhea and weight loss, sometimes requiring hospitalization. Onset of symptoms was months to years after initiation of olmesartan treatment. Intestinal biopsy specimens from 15 patients revealed villous atrophy and variable degrees of mucosal inflammation. Five patients had evidence of colonic inflammation. Most remarkably, a gluten-free diet did not resolve symptoms, whereas both marked symptomatic improvement and resolution of histopathologic findings occurred on withdrawal of olmesartan therapy.

We describe a patient who had been taking olmesartan for 3 years at which time small bowel histopathologic findings suggesting CD were documented, but symptoms of CD enteropathy were absent. This anecdotal observation suggests the possibility that olmesartan could be associated with histopathologic findings for a substantial period before the onset of enteropathy or alternatively that such histopathologic findings might persist for years without the onset of symptoms.

A 59-year-old man experienced mild, normochromic, normocytic anemia in 2007. Workup revealed an isolated vitamin B_{12} deficiency (172 pg/mL), which was ascribed to long-term ranitidine therapy for gastroesophageal reflux and which responded to oral vitamin B_{12} supplementation at 1000 μg/d. However, the anemia did not improve. The gastrin level was 41 pg/mL (reference range, <100 pg/mL); the intrinsic factor antibody test result was negative.

Coincidentally, the patient underwent upper gastrointestinal endoscopy for symptoms consistent with worsening gastric reflux. The only macroscopic finding was nodularity in the duodenal bulb consistent with prominent Brunner glands, which was attributed to acid wash. However, a biopsy specimen from the second portion of the duodenum revealed mild expansion of the lamina propria and increased intraepithelial lymphocytes (IELs) with no significant villous blunting, suggesting (but not diagnostic of) possible CD. The patient reported no diarrhea but had occasional mild constipation. He had a first-degree cousin with CD, but no other family members were known to have CD. Findings from a workup for CD were unremarkable, including negative tissue transglutaminase antibody results (0.9 AU, reference range, <7.0 AU), normal total IgA level (127 mg/dL; reference range, 50–500 mg/dL), normal vitamin K_1 level (1.16 ng/mL; reference range, 0.10-2.10), normal prothrombin time, and negative *Helicobacter pylori* antibody results. He was HLA-DQ2 positive but HLA-DQ8 negative.

Because the findings were unusual, a repeated upper endoscopy and a colonoscopy were performed in August 2010. The small bowel gross appearance was unchanged; the colonic examination findings were unremarkable. A small bowel biopsy specimen revealed increased IELs with mild villous blunting (interpreted as unchanged from the prior study); the colonic biopsy results were normal. The tissue transglutaminase antibody test result was again negative, and the total IgA level was normal.

A stool specimen for *Giardia* and *Cryptosporidium* immunoassays, obtained because of an episode of prolonged (6 weeks' duration) diarrhea during international travel 10 years previously, produced negative results. A trial of a gluten-free diet was considered, but the patient elected not to pursue this given the absence of symptoms, the uncertain diagnosis, and the logistical difficulties of dietary adherence during frequent domestic and international travel.

Hypertension had been diagnosed in 2003, and therapy with losartan was initiated. In 2004, losartan therapy was discontinued, and olmesartan therapy, 20 mg/d, was begun. Olmesartan therapy was well tolerated, and the hypertension was well controlled. On publication of the article by Rubio-Tapia et al, olmesartan was identified as a possible cause of the unusual findings. Olmesartan therapy will be discontinued, with monitoring of vitamin B_{12} levels and consideration for repeated upper gastrointestinal endoscopy.

Although Rubio-Tapia et al are careful to avoid claiming a proven causal relationship between olmesartan therapy and the observed spruelike enteropathy, the data are highly suggestive of more than just a coincidental association. The authors posit that the long interval between initiation of olmesartan therapy and onset of symptoms of enteropathy, as observed in their patients, could be consistent with cell-mediated immunity damage. They further suggest that a potential mechanism for the enteropathy could relate to inhibitory effects of angiotensin II receptor antagonists on transforming growth factor β action because transforming growth factor β is important in gut immune homeostasis.

Another interesting observation by the authors is that 68% of their patients

Exhibit L

UNITED STATES DISTRICT COURT

DISTRICT OF NEW JERSEY

IN RE: BENICAR (OLMESARTAN) PRODUCTS LIABILITY LITIGATION,	)	CIVIL NO. 15-2606(RBK)(JS)
THIS DOCUMENT RELATES TO ALL CASES	)	Judge Kugler

PROTECTED INFORMATION

- - - -

VIDEOTAPED EXPERT WITNESS TESTIMONY OF

DAVID A. KESSLER, M.D.

Held at the Law Offices of

Lieff, Cabraser, Heimann & Bernstein

275 Battery Street, San Francisco, California

Monday, February 6, 2017, 9:03 a.m.

- - - -

REPORTED BY: ELAINA BULDA-JONES, CSR #11720

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Also present:

Joseph Mourgos, videographer
Amy Klug, DSI (PRESENT TELEPHONICALLY)

THE VIDEOGRAPHER: We are back on the record. This marks the beginning of Disc No. 3 in the deposition of Dr. David Kessler. The time is 1:35 p.m.

BY MS. HUBBARD:

Q. Doctor, we are back from lunch, and let me direct you to Paragraph 77 of your report.

A. Yes, ma'am.

Q. This is the first paragraph in Section VII.

A. Right.

Q. And you talk about a review that you had done of MedWatch forms. And let me start with the time period. There is a time period of review of DSI MedWatch forms beginning in 2002.

And is that because that was the year of NDA approval?

A. Yes.

Q. And then the review was conducted of forms through 2012.

Did you have a particular month cutoff in 2012, or did you do it through the end of the year?

A. I have to go back and double-check. I have to go back and double-check the answer to that question. It was meant to be a ten-year period.

rechallenge, I think there was a discussion about what those terms were, I don't recall exactly, but the -- it was really any synonym that meant for rechallenge.

I have a couple of them written down, I think, somewhere.

Q. So my next question was going to be: Do you happen to have the list of -- the complete list of the terms?

A. I don't have -- I have run -- the -- it was the same sort of -- you know, the way I looked at -- in the 17 we looked at, you know, when you are looking for rechallenge, there are -- I took your report, and I'm sorry. I have mine.

Let's see what my -- at some point I made a list. I don't recall exactly. I have to go back and reconstruct that in my head.

But it was basically stop medicine, improvement, restart it, and then what happened. That's what I was looking for. So it was the restarting of the medicine, resume the medicine after symptoms abated, something like that.

Q. On the symptom abatement or improvement, was it a factor in narrowing the universe of AEs to be reviewed, was it a factor how long between

but Dosunmu had vomiting, so that's why I went with it.

So I'm sure I looked at the 2012 paper. I'm sure I -- I'm pretty sure I looked at that before I settled on these terms.

Q. All right. Before you settled on these terms, did you also look at Ford Parker's report to the FDA in September 2012?

A. Yes, I believe I looked at the 2012 and also the -- the December -- this is one that is actually misdated, I think, it's an '09 or 2010 report.

Q. The report by --

A. Caspard.

Q. -- Caspard on celiac?

A. Yes.

Q. Okay.

A. And that's why, in part, because celiac was a prevalent part of Dosunmu and -- both Dr. Dosunmu and Dr. Caspard. That's why that's included.

Q. For each of diarrhea, vomiting, and celiac, did you require that they also be accompanied by something else, like some secondary symptom, diarrhea cases accompanied by substantial

weight loss, or was this initial search of all diarrhea cases?

A. The most -- the thing that I insisted on, that was important to me to increase specificity, was seriousness.

So -- so while there is -- diarrhea is common and vomiting is common in the population, by restricting it to serious cases, there actually aren't that many -- I mean, there are cases.

But the cases that result in hospitalization can -- took it away from the common, our every day, to diarrhea and vomiting that also had the serious characteristic. So that was -- that was key to me, and essential.

So it's not all -- nor any celiac disease. There had to be a serious basis.

Q. Right. And that gets to one of the later factors, the third. But focusing just on the first factor, which was what symptoms were searched for, it -- if it was diarrhea, you weren't further limiting that symptom, in other words, it didn't have to be a certain kind of diarrhea?

We'll get to seriousness, but it --

A. The patient had to end up in the hospital --

Q. Right.

A. -- or there had to be a medical importance to that diarrhea. That's what I have limited it. Again, I --

Q. You used the seriousness to limit the scope of the symptom?

A. Yes. So -- so it goes into the -- well said, Counselor.

Q. Then let me ask you a little bit about your second criteria, which is here in Paragraph 78, which was, "Positive rechallenge was documented either through checked rechallenge box in Section C5 or the narrative in Section B5."

A. Right.

Q. All right. Let me ask you further about that. The box in C5 is a box that says, "Event abated after use stopped or dose reduced."

We can look together if that's helpful.

A. It has four checked -- it has four possible checkmarks.

Q. And it --

A. It has two for dechallenge and two for rechallenge.

Q. Exactly. And I think 5 is two of them and 8 is actually the other one, and so I want to make

it along.

A. That would be terrific.

Q. To start with, let me just have you look at the very first page.

A. Yes, ma'am.

Q. And if you look over on the right-hand side --

A. Yes.

Q. -- under C, C5, which is the section that you mention in your report related to your second criteria, says, "Event abated after use stopped or dose reduced."

A. Yes, ma'am.

Q. Do you see where I am?

A. Yes.

Q. Okay. And did you also look at whether C8, a box had been checked in C8. And that is -- 8 is, "Event reappeared after reintroduction."

A. Got you. So that's a mistake in my -- well caught, Counselor. I mean, I certainly mean C8 and -- there had to be both C5 and C8.

Q. Okay. So you are anticipating my next question.

Did -- if you were going to use the boxes, as opposed to the narrative, to identify a case of

rechallenge, did both boxes, C5 and C8, have to be checked?

A. So -- so in the -- for rechallenge, okay, that -- that you are -- and every time -- the criteria is specifically a positive rechallenge. I have not seen anything that has a rechallenge.

Rechallenge, by definition, means a dechallenge, so that's shortcut, okay. And every time I saw a rechallenge, I saw a dechallenge. That's my recollection.

Now, there's -- I will put a footnote there, because even though I designed these MedWatch reports, I will tell you -- I mean, I realize that there can be confusion, because when you look at the yes, what is your inclination, are you -- inclination to go to the right or to the left.

And it's not as perfect as we -- I had hoped for. But you had to have 8 checked yes. Okay.

And everything I -- when I looked at all of these, all -- and if 8 was checked yes, in fact, 5 was checked yes, unless there was something in the narrative that clearly indicated there was a rechallenge, right.

So you didn't necessarily have to have 8

checked if you had a narrative explaining the rechallenge, right.

Because, again, sometimes you could check the no and think you are checking the yes here if you look at the form. It is -- it is not perfect.

Q. But you -- if 8 was checked, you didn't necessarily have to have 5 checked because 8 presupposed 5; is that right?

A. I think so, yes. You don't -- you don't have -- if something reappeared after reintroduction, every time I have seen it, it disappeared on -- I mean, it abated after use.

And then the rechallenge was you did it again and -- after an event, after that episode abated or improved, is probably the right word.

Q. And that -- you are anticipating all my questions this afternoon.

And so in terms of event abated, if there was evidence of -- from the narrative, for example, of improvement without complete resolution of the condition while the patient was apparently off olmesartan, would that be sufficient to -- assuming on your part a dechallenge?

A. Yeah, so that is why in -- that's why I insisted both that I go through these to look

would be -- advantage your side, do you understand? I mean, right.

I mean, if there were more -- if there is things that I am not finding -- how do I say this? I'm just trying to think through.

Q. I'm -- I --

A. If there are more -- if there are more cases that I -- if there are more cases that I didn't find, then I am -- I am not helping -- not that I'm here to help or not, but just the bias cuts against Plaintiffs and advantages you.

And I don't care about any of that. So I -- my goal was to just to find new search criteria that were objective and find what I can.

If you could find others, you could find additional cases of rechallenge that I missed, please put them on the table, is what I mean.

Q. So, Doctor, I want to try to separate my questioning, if I can, into the search criteria to generate the group that turned out to be 62 AEs, that search criteria, from the review once those 62 were being reviewed.

So right now I just want to focus on the search criteria to generate that universe for review.

A. No -- there was no -- as long -- as long as there was evidence of a rechallenge, okay. And most people -- well, we can argue whether every additional dose I take is a rechallenge.

There is that debate in the -- in the scientific community, every time I take a medicine -- but that's not -- there was a period of dechallenge. Something has to improve, that was the criteria, and then there had to be a restart.

Q. Okay. And in the search criteria, then, the amount of time that passed before the event reappeared, that was not a limiting factor in the search criteria; do I understand that correctly?

MS. HAZAM: Objection. Vague and ambiguous. Form.

THE WITNESS: The -- the event reappeared -- so let's just understand this, you take a medicine, right, and you have certain symptoms. Now we have a dechallenge. I take it away, and there is an improvement.

So there is -- right, there is that time period. Okay. Then the next time is a rechallenge -- there is another administration, right.

MS. HAZAM: Object to the form. Foundation. Asked and answered.

THE WITNESS: So yes. That was not part of the -- the MedWatch report, okay, unless the company chose to follow up and arrange for that. That could have been done, I assume, if the company wanted to do something.

BY MS. HUBBARD:

Q. Okay. You are assuming that the company could have examined the patients?

A. No, no, no, what I am assuming, I mean, I am relying on company -- certainly there are MedWatch reports from clinical studies here, right, from ROADMAP.

Those patients were examined, right, per -- they were under the investigator ROADMAP that was done, so you could have -- so in certain instances you have the medical records of some of these patients when they're clinical study patients.

So, again, obviously we have to respect healthcare privacy, other things, but some of these are clinical study patients in --

Q. And some of them are postmarketing reports, correct?

MS. HAZAM: Will you please let him finish

Exhibit M

MAYO CLINIC

ORIGINAL ARTICLE

Olmesartan, Other Antihypertensives, and Chronic Diarrhea Among Patients Undergoing Endoscopic Procedures: A Case-Control Study

Ruby Greywoode, MD; Eric D. Braunstein, MD; Carolina Arguelles-Grande, MD; Peter H.R. Green, MD; and Benjamin Lebwohl, MD, MS

Abstract

Objective: To investigate a recent association between the use of the angiotensin receptor-blocker (ARB) olmesartan and a severe enteropathy resembling celiac disease.

Patients and Methods: We searched our endoscopy database for all outpatient esophagogastroduodenoscopy (EGD) or colonoscopy examinations in patients aged at least 50 years during the period January 1, 2007, to March 31, 2013. Cases were those whose examination indication was diarrhea, and controls were those whose examination indication was esophageal reflux (EGD) or colorectal cancer screening (colonoscopy). We compared cases with controls with regard to the proportion of those listing olmesartan among their medications. Secondary exposures were the proportion of those taking non-olmesartan ARBs or other antihypertensive medications. We also examined biopsy results to determine whether there were histologic changes associated with the use of olmesartan.

Results: We identified 2088 patients undergoing EGD and 12,428 patients undergoing colonoscopy meeting inclusion criteria. On multivariate analysis, there was no statistically significant association between olmesartan and diarrhea among those undergoing EGD (odds ratio, 1.99; 95% CI, 0.79-5.00) or colonoscopy (odds ratio, 0.63; 95% CI, 0.23-1.74). Review of pathology reports of the EGD and colonoscopy groups showed no association between the use of olmesartan and the histologic diagnosis of celiac disease ($P=.61$) or microscopic colitis ($P=1.0$), respectively.

Conclusion: Our findings suggest that neither olmesartan nor other ARBs were associated with diarrhea among patients undergoing endoscopy. The spruelike enteropathy recently associated with olmesartan is likely a rare adverse effect and milder presentations are unlikely.

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A number of recent reports in the literature have implicated olmesartan, an angiotensin II receptor-blocker (ARB) commonly prescribed for the treatment of hypertension, in the development of a severe form of chronic diarrhea and intestinal villous atrophy resembling celiac disease.[1] In an initial case series, 22 individuals were diagnosed with refractory celiac disease because of chronic diarrhea and villous atrophy on histology, although all lacked the diagnostic markers of celiac disease and derived no clinical improvement from a gluten-free diet. These individuals were observed to be taking olmesartan and experienced significant clinical and histological improvement with the cessation of the drug, suggesting a strong association between olmesartan and the development of a severe form of spruelike enteropathy.

A recent review of individuals with villous atrophy of unclear etiology also observed that a number of those originally considered to have unclassified sprue (negative celiac disease serologies despite evidence of villous atrophy on duodenal biopsy) were taking olmesartan.[2] As in the previous study, all these patients had symptomatic improvement after the discontinuation of the drug. Similarly, a case series of patients with collagenous sprue at the Mayo Clinic reported that of 30 patients with collagenous sprue, 27% had been taking olmesartan.[3] Although the diagnosis of celiac disease is made on duodenal biopsy, the finding of microscopic colitis (lymphocytic and/or collagenous colitis) in the large intestine is associated with a diagnosis of celiac disease. Thus, a positive association between microscopic colitis and the use of olmesartan could suggest a

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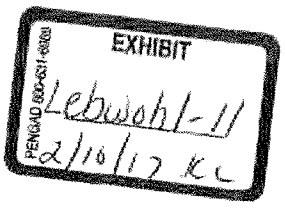

spectrum of histologic changes associated with the drug. In addition, lymphocytic colitis was present in 22% of the initial case series describing olmesartan-associated sprue-like enteropathy.

Another recent case report described similar findings of negative serologic markers despite mild villous atrophy in a patient taking olmesartan; however, unlike the previous reports, this patient exhibited no symptoms of diarrhea, suggesting that olmesartan may produce a spectrum of disease with preclinical or asymptomatic histologic changes.

It is unclear whether these cases described in the literature highlight a very rare reaction to olmesartan, or whether patients with severe disease represent the most clinically overt sample, with milder forms of olmesartan enteropathy left undetected. It is also unclear whether olmesartan alone is associated with this phenomenon or whether other members of its drug class share similar effects. We therefore performed a case-control study with the aim of investigating a possible association between diarrhea and the use of olmesartan among patients undergoing endoscopic procedures. As a secondary aim, we measured for associations between diarrhea and other antihypertensive medication exposures.

METHODS

Patients

Using an electronic endoscopy database, we identified all outpatient esophagogastroduodenoscopy (EGD) or colonoscopy examinations in patients aged at least 50 years during the 75-month period spanning the dates January 1, 2007, and March 31, 2013, at Columbia University Medical Center, a hospital-based endoscopy suite in New York City. As part of routine preendoscopy protocol, all patients were interviewed in person by a nurse and asked to provide a list of all their current medications (prescription as well as nonprescription). Cases were defined as those whose examination indication was listed as diarrhea, and controls were defined as those whose examination indication was esophageal reflux (in those undergoing EGD) or colorectal cancer screening (in those undergoing colonoscopy). We compared cases with controls with regard to the proportion of those who listed olmesartan among their medications. Secondary exposures were the proportion of those taking nonolmesartan ARBs or other antihypertensive medications. We used multivariate logistic regression, adjusting for age and sex, to quantify the association between these drug exposures and case status, that is, diarrhea.

To determine whether there were histologic changes associated with the use of olmesartan, we examined the biopsy results of both the EGD and the colonoscopy groups. We examined the upper endoscopy cases (ie, patients who presented for EGD because of diarrhea) to determine whether there were any diagnoses of celiac disease and whether there was an increased proportion of olmesartan use among those who underwent small intestinal biopsy during the procedure. To do so, we identified patients with celiac disease (either newly diagnosed or previously diagnosed) in this data set using a query for the *International Classification of Diseases, Ninth Revision* code for celiac disease (579.0) followed by manual review of the chart of each case with this diagnosis code. Using the search terms "microscopic colitis" or "lymphocytic colitis" or "collagenous colitis," we also manually reviewed the biopsy reports of colonoscopy cases (ie, patients who underwent colonoscopy because of diarrhea) to determine whether there was an increased proportion of microscopic colitis among patients taking olmesartan.

Statistical Analyses

For the primary outcome, we performed multiple logistic regression, controlling for age and sex, and calculated adjusted odds ratios (ORs) and their corresponding 95% CIs. All reported *P* values are 2-sided. We used SAS version 9.2. When comparing the use of olmesartan among cases diagnosed with celiac disease or microscopic colitis, we used the Fisher exact test. The Institutional Review Board at Columbia University Medical Center approved this study.

RESULTS

We identified 2088 patients undergoing EGD and 12,428 patients undergoing colonoscopy who met the inclusion criteria. Cases as defined by those undergoing endoscopy because of diarrhea were 393 (19%) in the EGD and 867 (7%) in the colonoscopy cohort (Table 1). Women composed 65% and 59% of the EGD and

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colonoscopy groups, respectively. Most patients were aged between 50 and 69 years (range, 50-93 y). The proportion of patients taking any antihypertensive was 46% (968/2088) of the patients in the EGD group and 42% (5267/12428) of the patients in the colonoscopy group. The use of olmesartan in particular was reported by 22 (1%) of the EGD and 83 (0.7%) of the colonoscopy study patients, while use of nonolmesartan ARB was reported by 228 (11%) of the EGD and 1048 (8%) of the colonoscopy patients.

Univariate (Table 2) and multivariate (Table 3) analyses demonstrated that there was no statistically significant association between the use of olmesartan and diarrhea among those undergoing EGD (multivariate OR, 1.99; 95% CI, 0.79-5.00) or colonoscopy (multivariate OR, 0.63; 95% CI, 0.23-1.74). Associations that reached statistical significance on multivariate analysis were an increased risk of diarrhea with older age (EGD OR for ≥70 y vs 50-59 y, 1.35; 95% CI, 1.01-1.80; colonoscopy OR, 2.22, 95% CI; 1.86-2.65) and female sex (EGD OR, 1.48; 95% CI, 1.16-1.90; colonoscopy OR, 1.69, 95% CI, 1.45-1.97). In addition, there was a decreased risk of diarrhea among EGD patients taking calcium channel blockers (OR, 0.61; 95% CI, 0.38-0.98) and angiotensin-converting enzyme inhibitors (OR, 0.67; 95% CI, 0.50-0.92) as well

TABLE 1. Characteristics of Study Patients

Characteristic	EGD (n=2088)	Colonoscopy (n=12,428)
Age (y)		
50-59	779 (37)	5621 (45)
60-69	763 (37)	4141 (33)
70+	546 (26)	2666 (21)
Sex		
Female	1364 (65)	7387 (59)
Male	724 (35)	5041 (41)
Procedure indication		
Diarrhea (cases)	393 (19)	867 (7)
Reflux (controls)	1695 (82)	—
CRC Screening (controls)		11,561 (93)
HTN medications		
None	1120 (54)	7161 (58)
Any	968 (46)	5267 (42)
Olmesartan	22 (1)	83 (0.7)
Any ARB	228 (11)	1048 (8)
Any ACEI	418 (20)	2235 (18)
HCTZ/chlorthalidone	218 (10)	1539 (12)
Beta blocker	404 (19)	2245 (18)
Calcium channel blocker	171 (8)	921 (7)

[a]ACEI = angiotensin-converting enzyme inhibitor; ARB = angiotensin II receptor-blocker; CRC = colorectal cancer; EGD = esophagogastroduodenoscopy; HCTZ = hydrochlorothiazide; HTN = hypertension.
[b]Values are No. (percentage).

TABLE 2. Univariate Analysis of Factors Associated With Diarrhea[a]

	EGD			Colonoscopy		
Factor	Diarrhea	Control	*P*	Diarrhea	Control	*P*
Age (y)			.38			<.001
50-59	139 (18)	640 (82)		290 (5)	5331 (95)	
60-69	140 (18)	623 (82)		297 (7)	3844 (93)	
70+	114 (21)	432 (79)		280 (11)	2386 (89)	
Sex						
Female	285 (21)	1079 (79)	<.001	608 (8)	6779 (92)	<.001
Male	108 (15)	616 (85)		259 (5)	4782 (95)	
Any antihypertensive	158 (16)	810 (84)	.006	369 (7)	4898 (93)	.91
No antihypertensive	235 (21)	885 (79)		498 (7)	6663 (93)	
Olmesartan	7 (32)	15 (68)	.12	4 (5)	79 (95)	.44
Any ARB	34 (15)	194 (85)	.51	87 (8)	961 (92)	.08
Any ACEI	60 (14)	358 (86)	.009	142 (6)	2093 (94)	.20
HCTZ/chlorthalidone	34 (16)	184 (84)	.20	84 (5)	455 (95)	.01
Beta blocker	74 (18)	330 (82)	.77	175 (8)	2070 (92)	.09
Calcium channel blocker	22 (13)	149 (87)	.04	66 (7)	855 (93)	.81

[a]ACEI = angiotensin-converting enzyme inhibitor; ARB = angiotensin II receptor-blocker; EGD = esophagogastroduodenoscopy; HCTZ = hydrochlorothiazide.
[b]Values are No. (percentage).
[c]Exposures meeting statistical significance.

TABLE 3. Multivariate Analysis of Factors Associated With Diarrhea

Factor	EGD		Colonoscopy	
	OR (95% CI)	P	OR (95% CI)	P
Age (y)				
50-59	1.0		1.0	
60-69	1.12 (0.86-1.45)	.41	1.44 (1.22-1.71)	<.001
70+	1.35 (1.01-1.80)	.04	2.22 (1.86-2.65)	<.001
Sex				
Female	1.48 (1.16-1.90)	.002	1.69 (1.45-1.97)	<.001
Male	1.0		1.0	
Any antihypertensive	0.72 (0.57-0.90)	.005	0.90 (0.76-1.04)	.14
Olmesartan	1.99 (0.79-5.00)	.14	0.63 (0.23-1.74)	.37
Any ARB	0.73 (0.49-1.09)	.12	1.17 (0.92-1.49)	.20
Any ACE	0.67 (0.50-0.92)	.01	0.89 (0.73-1.08)	.23
HCTZ/chlorthalidone	0.87 (0.58-1.30)	.49	0.66 (0.51-0.84)	<.001
Beta blocker	1.07 (0.80-1.43)	.66	1.11 (0.93-1.33)	.25
Calcium channel blocker	0.61 (0.38-0.98)	.04	0.97 (0.75-1.27)	.84

[a]ACEI = angiotensin-converting enzyme inhibitor; ARB = angiotensin II receptor blocker; EGD = esophagogastroduodenoscopy; HCTZ = hydrochlorothiazide.
[b]Exposures meeting statistical significance.

as among colonoscopy patients taking thiazide diuretics (OR, 0.66; 95% CI, 0.51-0.84).

Of the 393 patients who presented for upper endoscopy because of diarrhea, 70 (18%) had biopsy results consistent with celiac disease and 2 (0.5%) of those were taking olmesartan. When compared with EGD patients who presented because of diarrhea without a diagnosis of celiac disease on biopsy, there was no statistically significant association between the use of olmesartan and the diagnosis of celiac disease ($P=.61$) (data not shown).

Of the 867 patients who presented for colonoscopy because of diarrhea, 762 (88%) underwent biopsy and 59 of these had a diagnosis of microscopic colitis. None of the diagnoses of microscopic colitis, however, was associated with current use of olmesartan (data not shown). When compared with colonoscopy cases without a diagnosis of microscopic colitis on biopsy, there was no statistically significant association between the use of olmesartan and the diagnosis of microscopic colitis ($P=1.0$).

TABLE 4. Antihypertensive Use in EGD Cases With/Without Diagnosis of Celiac Disease on Biopsy

Antihypertensive	Diagnosis celiac disease (n=70)	No diagnosis celiac disease (n=323)
HTN medication, any	23 (33)	135 (42)
Olmesartan	2 (3)	5 (2)
Any ARB	2 (3)	32 (10)
Any ACE	11 (16)	49 (15)
HCTZ/chlorthalidone	7 (10)	27 (8)
Beta blocker	10 (14)	64 (20)
Calcium channel blocker	2 (3)	20 (6)

[a]ACEI = angiotensin-converting enzyme inhibitor; ARB = angiotensin II receptor blocker; HCTZ = hydrochlorothiazide; HTN = hypertension.
[b]Values are No. (percentage).
[c]$P=.6$.

DISCUSSION

In this case-control study, we sought to examine the recently described association between the use of olmesartan and chronic severe diarrhea using a large sample of patients presenting for endoscopy at a tertiary referral medical center. Previous data on the risk of diarrhea among individuals taking olmesartan come from the original trial comparing the use of olmesartan to placebo in patients with diabetes. Data from that trial suggested no increased gastrointestinal adverse effects of the drug; however, the risk of diarrhea with the use of olmesartan was not a primary end point of the study. To our knowledge, this is the first study to compare the rate of use of olmesartan and biopsy findings in patients with symptomatic chronic diarrhea vs asymptomatic individuals presenting for endoscopic evaluation.

We found that neither olmesartan nor other ARBs were associated with diarrhea among patients undergoing endoscopy. Other antihypertensives were negatively associated with diarrhea, possibly as a result of their known constipating effects. Analysis of the biopsy results of those patients who presented for endoscopy because of diarrhea similarly resulted in negative findings; there was no statistically significant association between patients whose biopsy results were consistent with a diagnosis of celiac disease or microscopic colitis and the use of olmesartan. Notably, most of the individuals in the initial case series who developed sprue-like enteropathy associated with the use of olmesartan were HLA DQ2 or DQ8 positive, suggesting potential predisposing factors in certain individuals; however, the underlying mechanism remains unknown.

Strengths of this study include the large sample size as well as the comprehensive and protocoled, direct, in-person solicitation of home medication use immediately preceding each endoscopic procedure. Limitations of this study include its retrospective nature, although it examines a large sample size for a rare event

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that may not be amenable to a prospective design. There was also a relatively small prevalence of use of olmesartan (0.7%-1%) among study patients, limiting the power of this analysis. Because the upper bound of our 95% CI was 5.00 in the EGD analysis and 1.74 in the colonoscopy analysis, a meaningful association between olmesartan and diarrhea may exist that was not detectable because of the relative rarity of use of olmesartan.

CONCLUSION

Our findings suggest that the spruelike enteropathy recently associated with olmesartan is a rare event and milder presentations causing diarrhea among substantial numbers of outpatients are unlikely. Future studies should focus on the mechanisms by which olmesartan causes severe spruelike enteropathy, and the identification of patient-related risk factors that predispose for this rare but serious outcome.

Abbreviations and Acronyms: **ARB** = angiotensin receptor-blocker; **EGD** = esophagogastroduodenoscopy; **OR** = odds ratio

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TABLE 5. Antihypertensive Use in Colonoscopy Cases With/Without Microscopic Colitis on Biopsy

Antihypertensive	Microscopic colitis (n=59)	No microscopic colitis (n=703)
HTN medication, any	24 (41)	296 (42)
Olmesartan	0 (0)	4 (0.6)
Any ARB	5 (8)	71 (10)
Any ACEI	13 (22)	109 (16)
HCTZ/chlorthalidone	6 (10)	63 (9)
Beta blocker	8 (14)	137 (19)
Calcium channel blocker	2 (3)	53 (8)

[a]ACEI = angiotensin-converting enzyme inhibitor; ARB = angiotensin II receptor-blocker; HCTZ = hydrochlorothiazide; HTN = hypertension.
[b]Values are No. (percentage).
[c]P=1.0.

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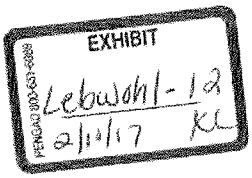

Sprue-like histology in patients with abdominal pain taking olmesartan compared with other angiotensin receptor blockers

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ABSTRACT

Aims A severe syndrome characterised by life-threatening diarrhoea and severe sprue-like histology has been described in patients taking the angiotensin receptor blocker (ARB) olmesartan. It is unknown whether there are any histopathological changes in patients without severe diarrhoea exposed to this medication. It is also unknown whether other ARBs cause sprue-like histology.

Methods Retrospective cohort study of patients with abdominal pain undergoing upper gastrointestinal endoscopy with duodenal biopsy who were taking ARBs. Patients taking olmesartan (n=20) and a non-olmesartan ARB (n=20) were compared with age and sex-matched controls. Histological features (classic sprue-like and other inflammatory changes) were analysed.

Results No single histopathological finding was significantly more common in olmesartan-using patients than controls. However, 10 of 20 olmesartan patients had one or more sprue-like histological features compared with 4 of 20 age-matched and sex-matched controls not taking ARBs (p=0.10). Patients taking ARBs other than olmesartan were not more likely than controls to have one or more of these sprue-like histological features (9/20 vs. 12/20, p=0.34).

Conclusions There were no statistically significant differences between olmesartan users with abdominal pain and controls for any single histopathological abnormality. However, there were trends towards significance for individual abnormalities as well as for a composite outcome of sprue-like changes. This raises the possibility that there is a spectrum of histological changes associated with olmesartan use.

INTRODUCTION

Olmesartan medoxomil is a commonly used antihypertensive medication, which acts by blocking angiotensin receptors. Recently, a series of cases were described in which 22 patients presented with debilitating diarrhoea and had a sprue-like enteropathy on histological examination due to olmesartan. The diarrhoea was so severe that 14 patients required hospitalisation and 4 required total parenteral nutrition. Serological testing for coeliac disease was negative in all cases and none improved with a gluten-free diet. All had biopsies, which showed severe sprue-like changes (villous atrophy, lamina propria inflammation and intraepithelial lymphocytosis (IEL)). Seven of the patients had collagenous sprue. All patients had dramatic improvement, with resolution of their diarrhoea following cessation of olmesartan.[1] As a major referral centre for coeliac disease, we have subsequently encountered a number of such cases and several other case series and reports have been published, which demonstrate similar clinical and histopathological findings.[2–15] At present, this adverse drug reaction is thought to be a rare occurrence. A recent case–control study did not show an association between olmesartan use and chronic diarrhoea in patients presenting for oesophagogastroduodenoscopy (OGD) or colonoscopy.[16]

While it is unusual to encounter severe villous atrophy in non-coeliac patients, milder changes which may overlap with sprue-like enteropathies (such mild or focal IEL) are common.[2 3 14] Medication reactions, particularly non-steroidal anti-inflammatory drugs, are commonly listed in the differential of such pathological findings.[15] Other drugs also enter the differential, but it is unknown whether olmesartan exposure should be considered when encountering such findings. It is also unknown whether other angiotensin receptor blockers (ARBs) may cause histopathological changes.

Because it is unclear whether the severe sprue-like enteropathy seen in a few patients taking olmesartan is the severe end of a spectrum of intestinal injury, we identified patients taking olmesartan who had undergone endoscopy for abdominal pain with duodenal biopsy and systematically studied the biopsies. We also identified patients with abdominal pain taking other ARBs who had duodenal biopsy and examined their biopsies to determine whether the changes were specific for olmesartan. We identified those patients whose indication for the procedure was abdominal pain to avoid those whose symptom was diarrhoea.

METHODS

We performed a retrospective cohort study using the electronic medical record of Columbia University Medical Center endoscopy unit (ProVation Medical Systems, Wolters Kluwer Health, New South Wales, Australia). This record includes all home medication use reported by outpatients undergoing OGD. This list of medications is ascertained by a trained nurse during an interview immediately preceding the procedure. We queried the medical record for patients in whom the indication for OGD was abdominal pain (self-reported, no formal diagnostic criteria employed) and identified 20 outpatients who listed olmesartan as one of their medications. We then matched each patient by age and gender to a control patient who did not report any ARB when listing his/her medications. Using the same process, we identified

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another 20 users of non-olmesartan ARBs and corresponding matched controls. We excluded all patients with a history of coeliac disease, inflammatory bowel disease or *Helicobacter pylori* infection (present or prior). In total, we identified 80 patients undergoing OGD for abdominal pain: 20 olmesartan users with 20 matched controls and 20 non-olmesartan ARB users with 20 matched controls. This study was approved by the Columbia University Medical Center Institutional Review Board.

Abnormalities that are seen in enteropathies that include coeliac disease and the sprue-like enteropathy of olmesartan including villous atrophy, crypt hyperplasia, increased IEL concentration, chronic lamina propria inflammation and increased subepithelial collagen deposition were evaluated on routine H&E-stained slides by a gastrointestinal pathologist who was blinded to the medication status (SML). The maximum IEL count in 100 epithelial cells was counted by routine H&E stain. In addition, increased crypt apoptosis (abnormal was considered more than 2 crypt apoptotic bodies in any 10 consecutive crypts or more than one apoptotic body per biopsy piece), active inflammation (defined as any extravascular neutrophils) and eosinophilia were also documented.

Statistical analysis

We compared the prevalence of each of the above histopathological findings among ARB users and their matched controls. We used the χ^2 and Fisher exact test when comparing proportions, and used the Mann–Whitney test when comparing IEL counts. After reviewing these comparisons, we subsequently performed a post-hoc analysis comparing ARB-exposed subjects with controls with regard to the composite outcome of one or more of the following findings: architectural abnormalities (villous atrophy or crypt hyperplasia), increased IEL or chronic inflammation. In this analysis, individuals who met one or more of these aforementioned criteria were collectively compared, via χ^2 testing, to those who met none of these criteria.

All p values reported are two-sided. We used SAS V9.3 (Cary, North Carolina, USA) for statistical calculations.

RESULTS

Among the 20 olmesartan users, the mean age was 59.5 years and 70% were women (table 1).

Among 20 non-olmesartan ARB users, the mean age was 58.5 years and 55% were women. The indication for OGD was abdominal pain in all cases and controls. When we compared duodenal biopsies of olmesartan users with controls, we identified no single histopathological finding that was significantly more frequent in either group (table 2).

However, there were variables and a composite outcome which showed trends towards significance. Of note, 10 of 20 olmesartan-exposed patients (50%) had one or more of the following sprue-like features: architectural distortion (villous atrophy and/or crypt hyperplasia), generalised increase in IEL and chronic inflammation (figure 1A–C). This compares with 4 of 20 control patients (10%, p=0.10). Regarding individual findings, olmesartan users had more positive findings than control patients for each variable investigated (other than increased subepithelial collagen which was not seen in any case or control), though none achieved statistical significance. Specifically, 25% of olmesartan users had foci of villous atrophy compared with 6% of control patients (p=0.33). The mean maximum IEL count was 13.7 in the olmesartan group compared with 10.6 for controls (p=0.09). Certain other features also were more common in olmesartan users than in control patients, but they too failed to reach statistical significance. The most notable of these was increased crypt apoptosis, which was seen in 25% of olmesartan users compared with 10% of controls (figure 1D).

We also compared duodenal biopsies from individuals taking ARBs other than olmesartan with patients taking no ARB. There were no statistically significant differences and no trends that suggested a similar effect (table 2).

DISCUSSION

Olmesartan is a widely prescribed ARB used in the management of hypertension. Rarely, patients taking this drug develop a life-threatening diarrheal illness with duodenal biopsies that reveal a severe enteropathy often with increased collagen deposition.[1] A study performed at our institution showed that over 10 years, 72 patients had been referred with a diagnosis of seronegative villous atrophy (negative coeliac disease serologies). The most common diagnosis in this group was seronegative coeliac disease (20 patients who had coeliac disease associated human leucocyte antigen haplotypes and responded to a gluten-free diet). The second most common diagnosis (n=19) was medication-related enteropathy. Sixteen patients had olmesartan exposure and had similar clinical and histological findings as described in the Mayo Clinic series. Eleven of the 16 olmesartan-exposed patients had increased subepithelial collagen.[5] Of considerable relevance to our study is a case reported by Talbot. The patient described was taking olmesartan, but did not have diarrhoea (presented with constipation). The patient had multiple endoscopies with biopsy. The first duodenal biopsy showed normal duodenal architecture

Table 1 Patient characteristics

	Olmesartan analysis		Other ARB analysis	
	Olmesartan users (n=20)	Matched controls (n=20)	Other ARB users (n=20) Losartan: 11 Valsartan: 3 Telmisartan: 3 Irbesartan: 2 Candesartan: 1	Matched controls (n=20)
Age (median, range)	59.5 (48–76)	59.5 (48–76)	58.5 (35–84)	58.5 (35–84)
Gender				
Male	6 (30)	6 (30)	9 (45)	9 (45)
Female	14 (70)	14 (70)	11 (55)	11 (55)

ARB, angiotensin receptor blocker.

Table 2 Histological features of olmesartan and other ARB users compared with controls

	Olmesartan analysis			Other ARB analysis		
	Olmesartan users (n=20) (%)	Matched controls (n=20) (%)	p Value	Other ARB users (n=20) (%)	Matched controls (n=20) (%)	p Value
Villous atrophy	4/16 (25)*	1/16 (6)	0.33	1/14 (7)*	2/19 (11)	1.0
Crypt hyperplasia	4/16 (25)*	2/17 (12)	0.40	3/14 (21)*	4/18 (22)	1.0
Mean maximum IEL count	13.7	10.6	0.09	13.0	18.5	0.35
Generalised IEL increase	4/20 (20)	2/20 (10)	0.67	2/20 (10)	6/20 (30)	0.24
Chronic inflammation	5/20 (25)	2/20 (10)	0.40	7/20 (35)	6/20 (30)	1.0
Eosinophilia	2/20 (10)	0/20 (0)	0.49	3/20 (15)	2/20 (10)	1.0
Neutrophilia	8/20 (40)	6/20 (30)	0.74	4/20 (20)	7/20 (35)	0.48
Increased crypt apoptosis	5/20(25)	2/20 (10)	0.40	6/20 (30)	8/20 (40)	0.74
One or more sprue-like features (architectural abnormalities, generalised increased IEL, chronic inflammation)	10/20 (50)	4/20 (20)	0.10	9/20 (45)	12/20 (60)	0.34

*Villous atrophy and crypt hyperplasia was not evaluated in 4 olmesartan cases and in 6 ARB cases due to poor orientation.
ARB, angiotensin receptor blocker; IEL, intraepithelial lymphocyte.

but had increased lamina propria lymphoplasmacytic inflammation and IEL. A subsequent biopsy was similar, although showed 'mild villous blunting.' Based on the reports previously described, this patient was taken off olmesartan despite the lack of significant symptoms.[16] It is intriguing to consider whether this patient would have developed the 'full-blown' clinical and histological syndrome if he had continued to take this agent. Also of particular relevance to this study is a case, which showed similar clinical and pathological characteristics as were described in the Mayo series of olmesartan patients in a patient taking another ARB, valsartan.[17]

To determine whether olmesartan usage was associated with intestinal damage, short of the severe sprue-like enteropathy, we identified patients with abdominal pain who were taking olmesartan or other ARBs and had a duodenal biopsy. We demonstrated a trend towards sprue-like enteropathic changes in individuals taking olmesartan compared with controls. The trend towards increased crypt apoptosis is interesting mechanistically, as certain other drugs known to cause intestinal damage often demonstrate this finding (e.g. mycophenolate mofetil).[18] These changes appear to be specific for olmesartan as there were none identified in those taking other ARBs.

This is the first study to our knowledge that investigates whether exposure to olmesartan or other ARBs is associated with histopathological abnormalities among outpatients

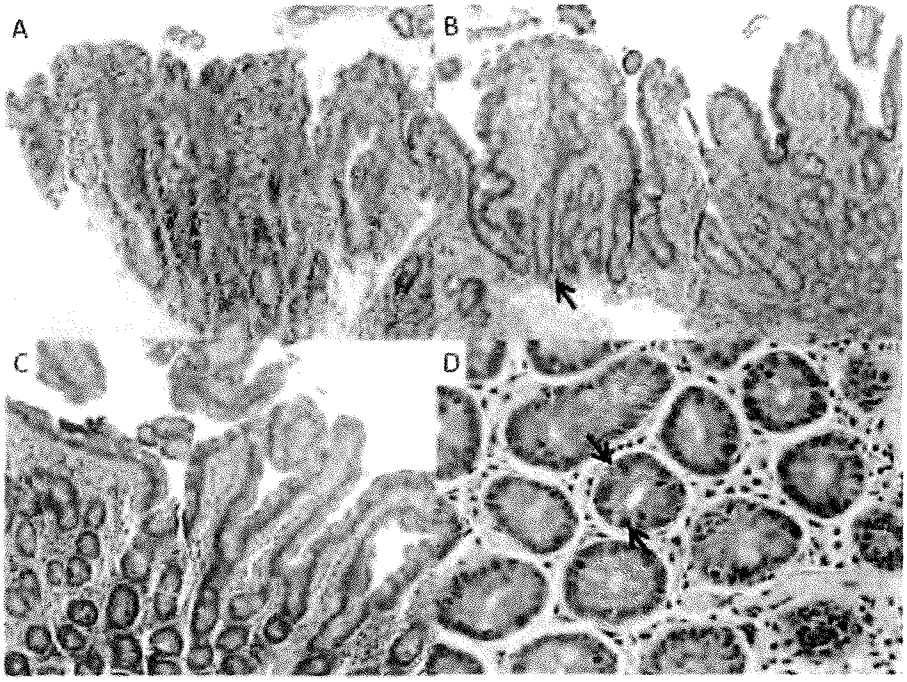

Figure 1 Highlighted findings in olmesartan users. (A) Representative photomicrograph of a small bowel biopsy from an individual showing one of several foci of villous atrophy, this particular case shows total villous atrophy but lacks intraepithelial lymphocytosis (H&E 200×). (B) A case with milder findings, including mild villous atrophy and focally pronounced crypt hyperplasia (arrow; H&E 100×). (C) This case had normal architecture, but a mild, generalised increase in intraepithelial lymphocytes (H&E 200×). (D) The case depicted in panel C also showed increased crypt apoptosis, including a crypt with 3–4 apoptotic bodies (arrows; H&E 600×).

undergoing duodenal biopsy. Our study has several limitations including its retrospective design, single centre setting and lack of information regarding duration of ARB use. We did not systematically exclude patients with known microscopic colitis; however, a post-hoc review showed that only 1 of 80 patients had microscopic colitis in our records (olmesartan user with no histopathological findings in our study). A larger sample size may have been useful, as it is possible that olmesartan causes a true increase in duodenal histopathological abnormalities but that our study was underpowered to detect this effect. Finally, we do not know whether any of the patients has subsequently discontinued olmesartan, and if so, if their abdominal pain has resolved.

This study raises the possibility that there may be a spectrum of injury associated with olmesartan use, apart from the severe syndrome that causes life-threatening diarrhoea. Further studies are needed to determine whether olmesartan use is associated with abdominal pain or other gastrointestinal symptoms and signs, as opposed to the well-characterised diarrhoea with sprue-like enteropathy. Future studies should follow-up the patients in this study to determine whether any of the olmesartan-exposed patients develop the severe enteropathic phenotype and if any of the histopathological variables we investigated are predictive thereof.

Take home messages

- This study raises the possibility that there is a spectrum of duodenal injury associated with olmesartan use.
- Angiotensin receptor blockers other than olmesartan are not associated with any histopathological findings in duodenal biopsies of patients with abdominal pain.
- Further studies are needed to determine whether olmesartan use is associated with abdominal pain and if the patients with the histopathological findings described here are at risk for developing the recently described severe sprue-like enteropathy.

Contributors SML: concept development, data collection, drafter of manuscript and guarantor of data. EDB: data collection and manuscript review. CA-G: concept development and manuscript review. GB: concept development and manuscript review. PG: concept development and manuscript review. BL: concept development, data analysis (statistics) and manuscript review.

Competing interests None.

Ethics approval Columbia University Medical Center Institutional Review Board.

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Exhibit N

Sprue-like histology in patients with abdominal pain taking olmesartan compared with other angiotensin receptor blockers

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ABSTRACT

Aims A severe syndrome characterised by life-threatening diarrhoea and severe sprue-like histology has been described in patients taking the angiotensin receptor blocker (ARB) olmesartan. It is unknown whether there are any histopathological changes in patients without severe diarrhoea exposed to this medication. It is also unknown whether other ARBs cause sprue-like histology.

Methods Retrospective cohort study of patients with abdominal pain undergoing upper gastrointestinal endoscopy with duodenal biopsy who were taking ARBs. Patients taking olmesartan (n=20) and a non-olmesartan ARB (n=20) were compared with age and sex-matched controls. Histological features (classic sprue-like and other inflammatory changes) were analysed.

Results No single histopathological finding was significantly more common in olmesartan-using patients than controls. However, 10 of 20 olmesartan patients had one or more sprue-like histological features compared with 4 of 20 age-matched and sex-matched controls not taking ARBs (p=0.10). Patients taking ARBs other than olmesartan were not more likely than controls to have one or more of these sprue-like histological features (9/20 vs. 12/20, p=0.34).

Conclusions There were no statistically significant differences between olmesartan users with abdominal pain and controls for any single histopathological abnormality. However, there were trends towards significance for individual abnormalities as well as for a composite outcome of sprue-like changes. This raises the possibility that there is a spectrum of histological changes associated with olmesartan use.

INTRODUCTION

Olmesartan medoxomil is a commonly used antihypertensive medication, which acts by blocking angiotensin receptors. Recently, a series of cases were described in which 22 patients presented with debilitating diarrhoea and had a sprue-like enteropathy on histological examination due to olmesartan. The diarrhoea was so severe that 14 patients required hospitalisation and 4 required total parenteral nutrition. Serological testing for coeliac disease was negative in all cases and none improved with a gluten-free diet. All had biopsies, which showed severe sprue-like changes (villous atrophy, lamina propria inflammation and intraepithelial lymphocytosis (IEL)). Seven of the patients had collagenous sprue. All patients had dramatic improvement, with resolution of their diarrhoea following cessation of olmesartan.[1] As a major referral centre for coeliac disease, we have subsequently encountered a number of such cases and several other case series and reports have been published, which demonstrate similar clinical and histopathological findings.[2–12] At present, this adverse drug reaction is thought to be a rare occurrence. A recent case–control study did not show an association between olmesartan use and chronic diarrhoea in patients presenting for oesophagogastroduodenoscopy (OGD) or colonoscopy.[13]

While it is unusual to encounter severe villous atrophy in non-coeliac patients, milder changes which may overlap with sprue-like enteropathies (such mild or focal IEL) are common.[2 14] Medication reactions, particularly non-steroidal anti-inflammatory drugs, are commonly listed in the differential of such pathological findings.[15] Other drugs also enter the differential, but it is unknown whether olmesartan exposure should be considered when encountering such findings. It is also unknown whether other angiotensin receptor blockers (ARBs) may cause histopathological changes.

Because it is unclear whether the severe sprue-like enteropathy seen in a few patients taking olmesartan is the severe end of a spectrum of intestinal injury, we identified patients taking olmesartan who had undergone endoscopy for abdominal pain with duodenal biopsy and systematically studied the biopsies. We also identified patients with abdominal pain taking other ARBs who had duodenal biopsy and examined their biopsies to determine whether the changes were specific for olmesartan. We identified those patients whose indication for the procedure was abdominal pain to avoid those whose symptom was diarrhoea.

METHODS

We performed a retrospective cohort study using the electronic medical record of Columbia University Medical Center endoscopy unit (ProVation Medical Systems, Wolters Kluwer Health, New South Wales, Australia). This record includes all home medication use reported by outpatients undergoing OGD. This list of medications is ascertained by a trained nurse during an interview immediately preceding the procedure. We queried the medical record for patients in whom the indication for OGD was abdominal pain (self-reported, no formal diagnostic criteria employed) and identified 20 outpatients who listed olmesartan as one of their medications. We then matched each patient by age and gender to a control patient who did not report any ARB when listing his/her medications. Using the same process, we identified

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another 20 users of non-olmesartan ARBs and corresponding matched controls. We excluded all patients with a history of coeliac disease, inflammatory bowel disease or *Helicobacter pylori* infection (present or prior). In total, we identified 80 patients undergoing OGD for abdominal pain: 20 olmesartan users with 20 matched controls and 20 non-olmesartan ARB users with 20 matched controls. This study was approved by the Columbia University Medical Center Institutional Review Board.

Abnormalities that are seen in enteropathies that include coeliac disease and the sprue-like enteropathy of olmesartan including villous atrophy, crypt hyperplasia, increased IEL concentration, chronic lamina propria inflammation and increased subepithelial collagen deposition were evaluated on routine H&E-stained slides by a gastrointestinal pathologist who was blinded to the medication status (SML). The maximum IEL count in 100 epithelial cells was counted by routine H&E stain. In addition, increased crypt apoptosis (abnormal was considered more than 2 crypt apoptotic bodies in any 10 consecutive crypts or more than one apoptotic body per biopsy piece), active inflammation (defined as any extravascular neutrophils) and eosinophilia were also documented.

Statistical analysis

We compared the prevalence of each of the above histopathological findings among ARB users and their matched controls. We used the χ^2 and Fisher exact test when comparing proportions, and used the Mann–Whitney test when comparing IEL counts. After reviewing these comparisons, we subsequently performed a post-hoc analysis comparing ARB-exposed subjects with controls with regard to the composite outcome of one or more of the following findings: architectural abnormalities (villous atrophy or crypt hyperplasia), increased IEL or chronic inflammation. In this analysis, individuals who met one or more of these aforementioned criteria were collectively compared, via χ^2 testing, to those who met none of these criteria.

All p values reported are two-sided. We used SAS V9.3 (Cary, North Carolina, USA) for statistical calculations.

RESULTS

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Among 20 non-olmesartan ARB users, the mean age was 58.5 years and 55% were women. The indication for OGD was abdominal pain in all cases and controls. When we compared duodenal biopsies of olmesartan users with controls, we identified no single histopathological finding that was significantly more frequent in either group (table 2).

However, there were variables and a composite outcome which showed trends towards significance. Of note, 10 of 20 olmesartan-exposed patients (50%) had one or more of the following sprue-like features: architectural distortion (villous atrophy and/or crypt hyperplasia), generalised increase in IEL and chronic inflammation (figure 1A–C). This compares with 4 of 20 control patients (10%, p=0.10). Regarding individual findings, olmesartan users had more positive findings than control patients for each variable investigated (other than increased subepithelial collagen which was not seen in any case or control), though none achieved statistical significance. Specifically, 25% of olmesartan users had foci of villous atrophy compared with 6% of control patients (p=0.33). The mean maximum IEL count was 13.7 in the olmesartan group compared with 10.6 for controls (p=0.09). Certain other features also were more common in olmesartan users than in control patients, but they too failed to reach statistical significance. The most notable of these was increased crypt apoptosis, which was seen in 25% of olmesartan users compared with 10% of controls (figure 1D).

We also compared duodenal biopsies from individuals taking ARBs other than olmesartan with patients taking no ARB. There were no statistically significant differences and no trends that suggested a similar effect (table 2).

DISCUSSION

Olmesartan is a widely prescribed ARB used in the management of hypertension. Rarely, patients taking this drug develop a life-threatening diarrheal illness with duodenal biopsies that reveal a severe enteropathy often with increased collagen deposition.[1] A study performed at our institution showed that over 10 years, 72 patients had been referred with a diagnosis of seronegative villous atrophy (negative coeliac disease serologies). The most common diagnosis in this group was seronegative coeliac disease (20 patients who had coeliac disease associated human leucocyte antigen haplotypes and responded to a gluten-free diet). The second most common diagnosis (n=19) was medication-related enteropathy. Sixteen patients had olmesartan exposure and had similar clinical and histological findings as described in the Mayo Clinic series. Eleven of the 16 olmesartan-exposed patients had increased subepithelial collagen.[2] Of considerable relevance to our study is a case reported by Talbot. The patient described was taking olmesartan, but did not have diarrhoea (presented with constipation). The patient had multiple endoscopies with biopsy. The first duodenal biopsy showed normal duodenal architecture

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Gender				
Male	6 (30)	6 (30)	9 (45)	9 (45)
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ARB, angiotensin receptor blocker.

Table 2 Histological features of olmesartan and other ARB users compared with controls

	Olmesartan analysis			Other ARB analysis		
	Olmesartan users (n=20) (%)	Matched controls (n=20) (%)	p Value	Other ARB users (n=20) (%)	Matched controls (n=20) (%)	p Value
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Crypt hyperplasia	4/16 (25)*	2/17 (12)	0.40	3/14 (21)*	4/18 (22)	1.0
Mean maximum IEL count	13.7	10.6	0.09	13.0	18.5	0.35
Generalised IEL increase	4/20 (20)	2/20 (10)	0.67	2/20 (10)	6/20 (30)	0.24
Chronic inflammation	5/20 (25)	2/20 (10)	0.40	7/20 (35)	6/20 (30)	1.0
Eosinophilia	2/20 (10)	0/20 (0)	0.49	3/20 (15)	2/20 (10)	1.0
Neutrophilia	8/20 (40)	6/20 (30)	0.74	4/20 (20)	7/20 (35)	0.48
Increased crypt apoptosis	5/20(25)	2/20 (10)	0.40	6/20 (30)	8/20 (40)	0.74
One or more sprue-like features (architectural abnormalities, generalised increased IEL, chronic inflammation)	10/20 (50)	4/20 (20)	0.10	9/20 (45)	12/20 (60)	0.34

*Villous atrophy and crypt hyperplasia was not evaluated in 4 olmesartan cases and in 6 ARB cases due to poor orientation.
ARB, angiotensin receptor blocker; IEL, intraepithelial lymphocyte.

but had increased lamina propria lymphoplasmacytic inflammation and IEL. A subsequent biopsy was similar, although showed 'mild villous blunting.' Based on the reports previously described, this patient was taken off olmesartan despite the lack of significant symptoms.[16] It is intriguing to consider whether this patient would have developed the 'full-blown' clinical and histological syndrome if he had continued to take this agent. Also of particular relevance to this study is a case, which showed similar clinical and pathological characteristics as were described in the Mayo series of olmesartan patients in a patient taking another ARB, valsartan.[17]

To determine whether olmesartan usage was associated with intestinal damage, short of the severe sprue-like enteropathy, we identified patients with abdominal pain who were taking olmesartan or other ARBs and had a duodenal biopsy. We demonstrated a trend towards sprue-like enteropathic changes in individuals taking olmesartan compared with controls. The trend towards increased crypt apoptosis is interesting mechanistically, as certain other drugs known to cause intestinal damage often demonstrate this finding (e.g. mycophenolate mofetil).[18] These changes appear to be specific for olmesartan as there were none identified in those taking other ARBs.

This is the first study to our knowledge that investigates whether exposure to olmesartan or other ARBs is associated with histopathological abnormalities among outpatients

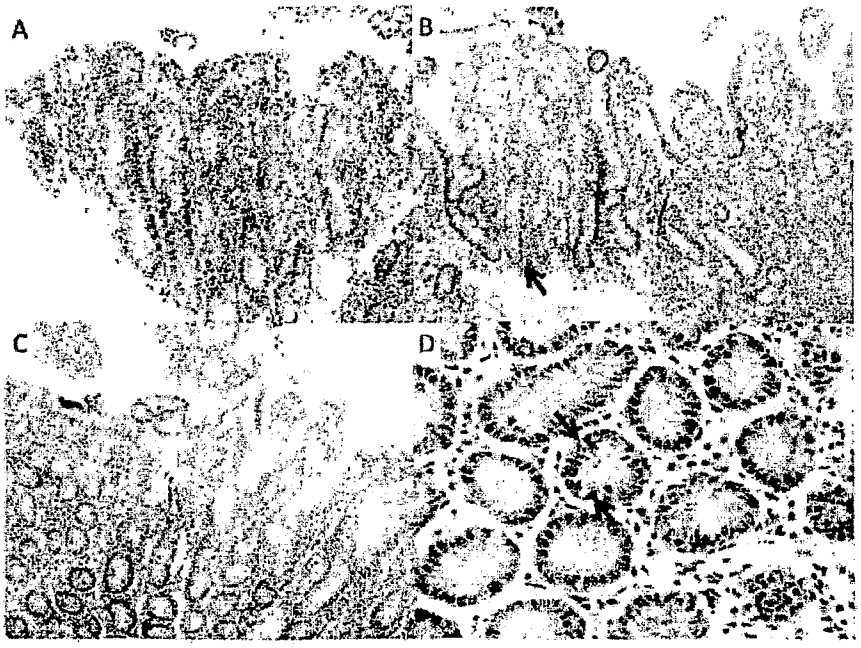

Figure 1 Highlighted findings in olmesartan users. (A) Representative photomicrograph of a small bowel biopsy from an individual showing one of several foci of villous atrophy, this particular case shows total villous atrophy but lacks intraepithelial lymphocytosis (H&E 200×). (B) A case with milder findings, including mild villous atrophy and focally pronounced crypt hyperplasia (arrow; H&E 100×). (C) This case had normal architecture, but a mild, generalised increase in intraepithelial lymphocytes (H&E 200×). (D) The case depicted in panel C also showed increased crypt apoptosis, including a crypt with 3–4 apoptotic bodies (arrows; H&E 600×).

undergoing duodenal biopsy. Our study has several limitations including its retrospective design, single centre setting and lack of information regarding duration of ARB use. We did not systematically exclude patients with known microscopic colitis; however, a post-hoc review showed that only 1 of 80 patients had microscopic colitis in our records (olmesartan user with no histopathological findings in our study). A larger sample size may have been useful, as it is possible that olmesartan causes a true increase in duodenal histopathological abnormalities but that our study was underpowered to detect this effect. Finally, we do not know whether any of the patients has subsequently discontinued olmesartan, and if so, if their abdominal pain has resolved.

This study raises the possibility that there may be a spectrum of injury associated with olmesartan use, apart from the severe syndrome that causes life-threatening diarrhoea. Further studies are needed to determine whether olmesartan use is associated with abdominal pain or other gastrointestinal symptoms and signs, as opposed to the well-characterised diarrhoea with sprue-like enteropathy. Future studies should follow-up the patients in this study to determine whether any of the olmesartan-exposed patients develop the severe enteropathic phenotype and if any of the histopathological variables we investigated are predictive thereof.

Take-home messages

- This study raises the possibility that there is a spectrum of duodenal injury associated with olmesartan use.
- Angiotensin receptor blockers other than olmesartan are not associated with any histopathological findings in duodenal biopsies of patients with abdominal pain.
- Further studies are needed to determine whether olmesartan use is associated with abdominal pain and if the patients with the histopathological findings described here are at risk for developing the recently described severe sprue-like enteropathy.

Contributors SML: concept development, data collection, drafter of manuscript and guarantor of data. EDB: data collection and manuscript review. CA-G: concept development and manuscript review. GB: concept development and manuscript review. PG: concept development and manuscript review. BL: concept development, data analysis (statistics) and manuscript review.

Competing interests None.

Ethics approval Columbia University Medical Center Institutional Review Board.

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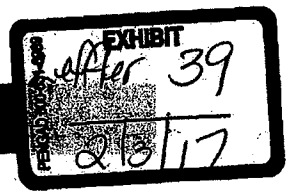
Exhibit O



Comparative Effectiveness of Olmesartan and Other Angiotensin Receptor Blockers in Diabetes Mellitus

Retrospective Cohort Study

Raj Padwal, Mu Lin, Mahyar Etminan, Dean T. Eurich

Abstract—Olmesartan has been linked with increased risk of cardiovascular mortality and sprue-like enteropathy. We compared outcomes between olmesartan and other angiotensin receptor blockers in a large clinical registry of patients with diabetes mellitus. A retrospective cohort analysis using nationwide US-integrated insurance and laboratory claims was performed in 45 185 incident diabetic angiotensin receptor blocker users, including 10 370 (23%) olmesartan users. Hazard ratios were computed using time-dependant Cox models adjusted for sociodemographic characteristics, comorbidities, laboratory data, drug use, healthcare utilization, and the propensity to receive olmesartan. Blood pressure data were unavailable. Subjects were followed up for 116 721 patient-years. The primary end point was all-cause hospitalization or all-cause mortality and occurred in 10 915 (24%) patients. Average age was 54.3±9.6 years, 52% were men, 17% had cardiovascular disease, and 10% chronic kidney disease. Compared with other angiotensin receptor blockers, the adjusted hazard for olmesartan was 0.99 (95% confidence interval, 0.94–1.05) for all-cause hospitalization and mortality; 0.90 (0.62–1.30) for all-cause mortality; 0.99 (0.94–1.05) for all-cause hospital admission; 0.88 (0.78–1.00) for cardiovascular disease–related admission, and 1.09 (0.98–1.20) for gastrointestinal disease–related hospitalization in the overall cohort. Olmesartan use was associated with an adjusted hazard for the primary outcome of 1.11 (0.99–1.24) in subjects with history of cardiovascular disease and 1.21 (1.04–1.41) in subjects with chronic kidney disease. In conclusion, there is no robust signal for harm with olmesartan use. Risk may be increased in kidney disease; thus, given the widespread availability of alternate agents, olmesartan should be used with caution in this subgroup pending further study. (***Hypertension.*** **2014;63:977-983.)** • **Online Data Supplement**

Key Words: angiotensin receptor antagonists ■ cardiovascular diseases ■ comparative effectiveness research ■ hospitalization ■ mortality ■ olmesartan

Olmesartan, an angiotensin II type 1 receptor antagonist (ARB) first approved in 2002, is commonly used for the treatment of hypertension.[1] Despite being the seventh ARB approved by the Food and Drug Administration and despite a lack of hard outcome trial data supporting its use, olmesartan is widely prescribed, with estimated worldwide sales of 2 billion US dollars in 2009.[2] Two placebo-controlled randomized controlled trials examining the efficacy of olmesartan in delaying onset/progression of renal disease in patients with diabetes mellitus, Randomized Olmesartan and Diabetes Microalbuminuria Prevention (ROADMAP) and Olmesartan Reducing Incidence of End Stage Renal Disease in Diabetic Nephropathy (ORIENT), have been recently published.[3,4] In both trials, cardiovascular mortality was increased in subjects randomized to olmesartan treatment. In ROADMAP, cardiovascular deaths occurred in 15 (0.7%) olmesartan-treated subjects and 3 (0.1%) placebo-treated subjects (P=0.01). In subjects with pre-existing cardiovascular disease taking olmesartan, 11 cardiovascular deaths occurred compared with 1 in subjects assigned to placebo. In ORIENT, 10 (3.5%) subjects receiving olmesartan died of cardiovascular causes compared with 3 (1.1%) placebo-treated subjects (P>0.05). Although these data raise concerns, they do not definitively prove harm because cardiovascular death was not a primary end point, the absolute number of cardiovascular events was low in both studies, and nonfatal cardiovascular events were not significantly different between study arms in ROADMAP (81 [3.6%] for olmesartan versus 91 [4.1%] for placebo; P=0.31).

After undertaking a safety review of olmesartan in 2011, the US Food and Drug Administration determined that the benefits of the drug outweighed its potential risks in patients with hypertension but advised against use of olmesartan for

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delaying or preventing renal disease and underscored the need for more postmarketing surveillance.[5] In 2013, following case reports describing a potential association between olmesartan and sprue-like enteropathy, the Food and Drug Administration issued a second warning and announced plans to conduct further safety reviews.[6]

The objective of this study was to provide further postmarketing assessment of the comparative effectiveness and safety of olmesartan. Specifically, we assessed the effect of olmesartan therapy compared with other ARBs on overall mortality and cause-specific hospitalization and sought to quantify absolute event rates. Given prior evidence, we hypothesized that olmesartan use would increase the risk of mortality or hospitalization relative to other ARBs in patients with diabetes mellitus, and that this risk increase would be highest in patients with pre-existing cardiovascular disease and chronic kidney disease (CKD; ie, high-risk subgroups).

Methods

We performed a population-based retrospective cohort study using an anonymized large US claims and integrated laboratory database containing information on employed, commercially insured patients with dependants from all 50 states (Clinformatics Data Mart, Optum, Life Sciences). The database has been used in multiple previous studies, contains >13 million annual lives.[7–10] We analyzed patient-level, clinically rich, deidentified longitudinal data, including administrative and demographic information (sex, age, type of insurance plan, eligibility date, and income); billable medical service inpatient, outpatient, and medical procedure claims (deidentified physician and facility identifier, date and place of service, cost of service, admission and discharge dates, procedure, and diagnosis codes); and laboratory test results and pharmacy claims data (deidentified prescribing physician, drug dispensed based on national drug codes, quantity and date dispensed, drug strength, days' supply, and cost of service). *International Classification of Diseases, Ninth Revision, Clinical Modification* (*ICD-9-CM*) clinical and procedure codes were used, and data were cleaned and analyzed using protocols compliant with the Health Insurance Portability and Accountability Act.

Research ethics review board approval to conduct this study was obtained from the University of Alberta and the New England Institutional Review Board. The procedures followed were in accordance with institutional guidelines.

Cohort Selection

An inception cohort of 114 010 new ARB users with diabetes mellitus aged ≥20 years and identified between January 1, 2004 and December 31, 2009 was created. The date of the first ARB prescription was designated as the index date. New users were individuals who did not have a prior prescription claim for any ARB for ≥1 year before their index date. We limited inclusion to subjects with ≥1 year of baseline data enrolled in a commercial medical insurance plan (Figure 1). Subjects were followed up until death, termination of medical insurance, or December 31, 2010 (study end) providing a maximum follow-up of 6 years. A priori, we decided to exclude users who crossed over from olmesartan to another ARB (or vice versa) during the follow-up period (n=3257). Mortality was ascertained by linking to the US national death index file.[11] This is a highly valid and reliable method, with >98% sensitivity when social security number data are available.[12]

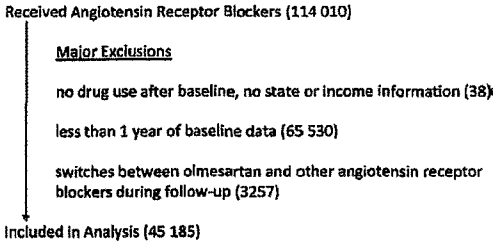

Figure 1. Inclusions and exclusions.

The primary outcome was all-cause hospital admission or death. This composite outcome was analyzed using time-to-first event (eg, either admission date or date of death) as the dependent variable. Each component of this composite end point was also analyzed separately. Cause-specific mortality was not available. Other secondary end points included cardiovascular-related hospital admissions (*ICD-9-CM* codes 410, 411.1, 428, 430–438), the combined end point of cardiovascular-related hospital admission or all-cause mortality, gastrointestinal-related hospital admissions (*ICD-9-CM* codes 530–579), and admissions related to noninfective enteritis and colitis (*ICD-9-CM* codes 555–558). Patients were censored if they did not have an outcome of interest and reached study end (December 31, 2010) or their insurance was terminated.

Analyses

Time-varying Cox proportional hazards regression was used to estimate the effect of exposure to olmesartan (relative to all other ARBs) on each outcome. Time zero was set at index date.[13] The days' supplied field in the prescription drug dispensations database was used as a proxy for the expected duration of each prescription and was used to compute time-varying drug exposure.[14] We assumed that subjects were exposed to the drug of interest unless prescription refills were not obtained for 2 consecutive days' supplied periods. If drug discontinuation occurred, subjects were classified as unexposed from the end of the first consecutive days' supplied period to the end of the study or until they restarted the drug. In this time-varying primary analysis, outcome events were attributed to a given drug if the event occurred while the subject was exposed; no legacy or carryover effects from remote exposure were assumed.

Covariates

In addition to using time-varying exposure models to limit potential bias, additional potential confounders were included in the Cox regression models as time fixed baseline variables. These included age, sex, socioeconomic status (type of medical insurance and median household income according to the 2010 US census),[15] cardiovascular comorbidities, clinical laboratory data (glycohemoglobin, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, estimated glomerular filtration rate [according to the Modified Diet in Renal Disease calculation: ≥90, 89.9–60, 59.9–30, <30 mL/min], albuminuria, and hemoglobin concentrations), and prescription drugs (eg, antidiabetic agents, antiplatelet drugs, anticoagulants, statins, calcium channel blockers, β-blockers, angiotensin-converting enzyme inhibitors, renin inhibitors, diuretics, and nitrates). For patients who did not have specific clinical laboratory data measured, we used the missing indicator approach for all analyses.[16]

To further control for baseline comorbidity and illness, we included an Adjusted Clinical Groups score in the model. This single comorbidity score is derived from the Johns Hopkins Adjusted Clinical Groups score system (Version 9)[17] and is weighted by 32 adjusted diagnostic groups. It performs equally to or better than the Charlson and Elixhauser comorbidity scores.[18] In addition, we adjusted for the total number of hospital admissions in the year before the index date, the total number of chronic conditions at baseline, frailty (any occurrence of malnutrition, abnormal weight loss, morbid obesity, dementia, falls, and decubitus ulcer),[17] and the time-varying propensity to receive olmesartan. For the latter, we computed the updated propensity or probability of receiving olmesartan every 3 months throughout the follow-up period.[19] This propensity score was entered into the model as a continuous probability score that was based on ≈60 variables, including demographic variables (age, sex, age–sex interaction, state, and type of insurance), socioeconomic factors (income), comorbidities, health service use, laboratory data, markers of frailty, and drug treatments. A full list of model covariates and variables included in the propensity score is available on request.

Subgroup and Sensitivity Analyses

Subgroup analyses were performed in subjects with a baseline history of cardiovascular disease and with CKD (defined as an estimated glomerular filtration rate <60 mL/min). A sensitivity analysis in which we repeated primary analysis comparing olmesartan with all other ARBs but censored subjects who switched from one ARB class to another (instead of excluding them) was also performed.

A dose–response analysis and an analysis comparing olmesartan with individual ARBs were also performed. Further methodological details are provided in the online-only Data Supplement.

Results

Of 114 010 ARB users, the final cohort comprised 45 185 subjects (Figure 1). Mean age was 54.3 (SD, 9.6) years, 52% were men, 17% had a history of cardiovascular disease, 13% had diabetes mellitus–related complications, and 10% had CKD (Table 1). We identified 10 370 (23%) olmesartan users and 34 815 (77%) who used other ARBs during the follow-up period. Additional baseline characteristics of the study population are summarized in Table 1. The prevalence of concomitant comorbidities was either equal between groups or lower in olmesartan users compared with users of other ARBs. One exception was hypertension, which was more common in olmesartan users. The average daily ARB doses prescribed during the follow-up period were olmesartan 22.1 mg, losartan 52.1 mg, valsartan 110.5 mg, telmesartan 41.9 mg, eprosartan 424.2 mg, irbesartan 145.9 mg, and candesartan 14.1 mg.

Subjects were followed up for 116 721 patient-years (median duration, 2.3 years [interquartile range, 1.1–3.8 years]). The primary composite end point occurred in 10 915 (24%) subjects; 10 836 (24%) subjects experienced ≥1 hospital admission and 458 (1%) died (Table 2).

The crude incidence rates of all-cause hospital admission or all-cause mortality were lower in olmesartan users compared with other ARBs (Table 2). However, after time-varying, multivariable adjustment was performed, the relative hazard of the primary composite end point was similar in olmesartan users (adjusted hazard ratio [aHR], 0.99; 95% confidence interval, 0.94–1.05; Table 2; Figure 2). In addition, compared with other ARB users, aHRs in olmesartan users were 0.90 (0.62–1.30) for all-cause mortality; 0.99 (0.94–1.05) for all-cause hospital admission; and 0.88 (0.78–1.01) for cardiovascular disease–related hospitalization (Table 2).

The covariate-aHR of gastrointestinal disease–related hospitalization was 1.09 (0.98–1.20) for olmesartan users compared with other ARB users and the aHR for admissions related to noninfective enteritis and colitis was 1.21 (0.87–1.69; Table 2).

Subgroup Analyses

Results in high-risk subjects are summarized in Table 3. In subjects with pre-existing cardiovascular disease, the aHR for the primary outcome was 1.11 (0.99–1.24) in olmesartan users. The aHR for the primary outcome was increased in olmesartan users with CKD (aHR, 1.21 [1.04–1.41]).

Sensitivity Analysis Censoring Rather Than Excluding ARB Switchers

In this analysis (n=48 475), the aHRs comparing olmesartan with all other ARBs for the primary outcome were 1.02 (95% confidence interval, 0.97–1.08) in the overall cohort,

Table 1. Baseline Characteristics of Olmesartan and Other ARB Users

	Olmesartan Users (n=10 370)	Other ARB Users (n=34 815)	*P* Value
Age, y	53.7±9.3	54.4±9.7	<0.0001
Sex			0.3709
Men	5472 (53)	18 197 (52)	
Women	4898 (47)	16 618 (48)	
Annual income, US dollars	48 034±6052	48 380±6237	<0.0001
Type of insurance			<0.0001
Point of service	6003 (58)	19 722 (57)	
Exclusive provider	1901 (18)	5956 (17)	
Preferred provider	889 (9)	3399 (10)	
Health maintenance	1463 (14)	5267 (15)	
Independent	108 (1)	455 (1)	
Other	6 (0)	16 (0)	
Clinical parameters at baseline			
Adjusted Diagnostic Groups Comorbidity Score	11±9	13±10	<0.0001
History of CV disease			
Ischemic heart disease	1425 (14)	6400 (18)	<0.0001
Heart failure	333 (3)	2065 (6)	<0.0001
Myocardial infarction	89 (1)	645 (2)	<0.0001
Dyslipidemia	6270 (60)	20 823 (60)	0.2339
Hypertension	9067 (87)	38 745 (83)	<0.0001
Arrhythmia	535 (5)	2463 (7)	<0.0001
Valvular heart disease	400 (4)	1698 (5)	<0.0001
eGFR categories, mL/min			<0.0001
<30	50 (0.5)	388 (1)	
30 to <60	824 (8)	3313 (10)	
60 to <90	5768 (56)	18 564 (53)	
≥90	3728 (36)	12 500 (36)	
Albuminuria (≥5 g/dL)	612 (6)	2533 (7)	<0.0001
Total cholesterol, mg/dL	192±46	190±46	0.0023
Triglycerides, mg/dL	181±174	180±195	0.7589
HDL, mg/dL	47±13	48±14	0.1175
LDL, mg/dL	112±37	109±37	0.0008
A1c, %	7.1±1.7	7.3±1.8	<0.0001
Hemoglobin, g/dL	14.1±1.6	13.9±1.6	<0.0001
Medication use			
Metformin	3404 (32)	11 988 (34)	0.0024
Sulfonylureas	1956 (19)	7525 (22)	<0.0001
Thiazolidinediones	1579 (15)	5951 (17)	<0.0001
Insulin	1032 (10)	4511 (13)	<0.0001
RAS blocker (ACE inhibitor or direct renin inhibitor)	4148 (40)	13 509 (39)	0.0282
Statins	4026 (39)	13 689 (39)	0.3641
β-Blockers	2610 (25)	9384 (27)	0.0003
Dihydropyridine CCB	1805 (17)	5494 (16)	<0.0001
Non-dihydropyridine CCB	602 (6)	2119 (6)	0.2906
Nitrates	336 (3)	1545 (4)	<0.0001

(*Continued*)

Table 1. Continued

	Olmesartan Users (n=10370)	Other ARB Users (n=34815)	*P* Value
Diuretics	2641 (25)	8574 (24)	0.0820
Anticoagulants	227 (2)	1073 (3)	<0.0001
Antiplatelets	459 (4)	2157 (6)	<0.0001
Healthcare utilization			
Inpatient hospitalization in year before index?			<0.0001
0	9473 (91)	30438 (87)	
1	744 (7)	3404 (10)	
≥2	153 (1)	973 (3)	
Frailty	429 (4)	1536 (4)	0.2282
Chronic conditions in year before index date			<0.0001
≤1	1900 (18)	6373 (18)	
2–5	6653 (64)	20540 (59)	
≥5	1817 (18)	7902 (22)	
Medication possession ratio for DM-related medications	0.44±0.7	0.47±1.0	0.0005

Data are n (%) or mean±SD. A1c indicates hemoglobin A1c; ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; CCB, calcium channel blocker; CV, cardiovascular; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; and RAS, renin angiotensin system.

1.07 (0.93–1.24) in pre-existing cardiovascular disease, and 0.91 (0.82–1.01) in CKD.

Dose–Response Sensitivity Analyses

Results of the dose–response analysis are summarized in Table S1 in the online-only Data Supplement. In the overall cohort and in the cardiovascular disease subgroup, higher doses of olmesartan were associated with significantly increased risk for the primary outcome. The dose–response analyses for valsartan showed similar results to olmesartan. However, the dose–response analysis for losartan did not show increasing risk with higher doses (Table S1).

Results of the analysis comparing individual ARB agents are summarized in Table S2. In this sensitivity analysis, olmesartan was not consistently associated with the highest risk of harm. Few statistically significant differences were found between agents. Exceptions were that losartan was associated with a borderline statistically significant increase in the primary end point in subjects with cardiovascular disease, and the other ARBs (candesartan, eprosartan, and irbesartan) were associated with a lower risk for the primary end point in the CKD subgroup only (Table S2). In both cases, this result was driven by significant reductions in hospitalizations but not mortality (data not shown).

Discussion

In this analysis of a clinically rich data set encompassing >45000 patients with diabetes mellitus, after extensive multivariable adjustment, we found that olmesartan use compared with other ARB use was not associated with an increased risk of hospitalization or all-cause mortality in the overall cohort. In fact, there was a trend toward a lower relative hazard for cardiovascular hospitalizations. However, in the higher-risk subjects (those with pre-existing cardiovascular disease or CKD), the aHRs for this primary end point were increased, and this risk increase was statistically significant in subjects with CKD (however, this finding was not robust to sensitivity analysis). The increased risk was primarily driven by an increase in the relative hazard of all-cause hospitalization. When we examined cause-specific hospitalization, we found no statistically significantly increased risk for cardiovascular disease–related and gastrointestinal disease–related hospitalization. A dose–response analysis of olmesartan found an increased risk for the primary end point in the overall cohort and in subjects with cardiovascular disease. However, similar findings were observed in a dose–response analysis for valsartan (but not losartan). This suggests that higher doses might have been a marker of increased risk rather than a causative factor. Finally, in the agent-specific analysis, olmesartan was not consistently associated with the highest risk, and few statistically significant differences between agents were found. In aggregate, our results do not demonstrate a robust signal for harm with olmesartan use in patients with diabetes mellitus, with the possible exception of diabetes mellitus with CKD.

One prior, large retrospective cohort analysis comparing olmesartan with other ARBs has been published.[20] This study of 118700 subjects enrolled in a single US national healthcare plan reported that olmesartan use was associated with a lower risk of cardiac events compared with valsartan,

Table 2. Outcome Comparisons in Olmesartan Users vs Users of All Other Angiotensin Receptor Blockers

Outcome	Time at Risk (Person-Years)	Events , n (%)	Unadjusted HR (95% CI)	Adjusted HR (95% CI)	*P* Value
All-cause hospitalization or mortality	16040	1686 (16)	0.87 (0.83–0.92)	0.99 (0.94–1.05)	0.89
All-cause mortality	18310	35 (0.3)	0.67 (0.47–0.97)	0.90 (0.62–1.30)	0.56
All-cause hospitalization	16040	1678 (16)	0.87 (0.83–0.92)	0.99 (0.94–1.05)	0.91
CV disease–related hospitalization	17951	311 (3)	0.67 (0.59–0.75)	0.88 (0.78–1.00)	0.051
GI disease–related hospitalization	17647	498 (5)	0.98 (0.88–1.08)	1.09 (0.98–1.20)	0.10
Noninfective enteritis and colitis-related admissions	18247	46 (0.4)	1.05 (0.75–1.47)	1.21 (0.87–1.69)	0.26

Models adjusted for age, sex, socioeconomic status, cardiovascular comorbidities, clinical laboratory data, prescription drugs, Adjusted Clinical Groups score, total number of hospital admissions in the year before the index data, total number of chronic conditions at baseline, frailty, and the time-varying propensity to receive olmesartan. CI indicates confidence interval; CV, cardiovascular; GI, gastrointestinal; and HR, hazard regression.

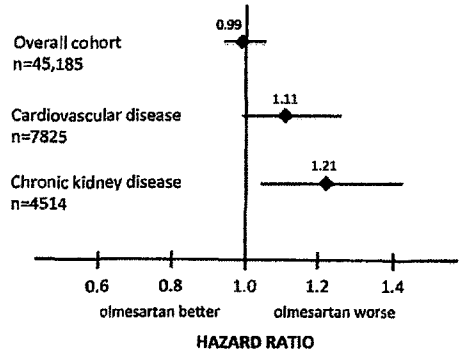

Figure 2. Adjusted hazard ratios and 95% confidence intervals for all-cause hospital admission or all-cause death according to olmesartan exposure.

irbesartan, and losartan. A limitation of this analysis was that olmesartan was prescribed to lower risk individuals, and no propensity score adjustment was used. In addition, the study population was broad and not limited to subjects with type 2 diabetes mellitus and no high-risk subgroup analyses were performed. Thus, although these findings are broadly consistent with the results of our study, they are not directly comparable because of differences in study populations and methodologic approaches.

Olmesartan is a third-generation high-affinity ARB with a 12- to 15-hour half-life that is prescribed once daily.[1,21] It is available as a dual combination product with hydrochlorothiazide or amlodipine and as a triple combination preparation with both of these agents.[21] No clinical trials demonstrating reductions in cardiovascular morbidity and mortality outcomes have been published.[22] The ongoing 1147 patient Supplemental Benefit of an Angiotensin Receptor Blocker in Hypertensive Patients with Stable Heart Failure Using Olmesartan (SUPPORT) trial is evaluating the efficacy of olmesartan compared with non-ARB antihypertensives in reducing a composite of all-cause mortality, nonfatal acute myocardial infarction, nonfatal stroke, and hospital admissions for heart failure.[23] Results are expected in 2013 to 2014.

Potential mechanisms to explain the association between olmesartan use and increased hospitalizations are not known. A J-curve mechanism resulting from excessive diastolic blood pressure lowering has been proposed to explain increased cardiovascular risk with olmesartan use in placebo-controlled studies.[3,4] Notably, previous studies comparing olmesartan with either placebo or atenolol therapy have reported that olmesartan leads to comparatively favorable improvements in such surrogate cardiovascular end points as vascular remodeling, endothelial dysfunction, inflammatory biomarkers, and atherosclerotic plaque volume.[24–26] In addition, olmesartan has been proposed to possess potential cardiovascular benefits compared with other ARBs because it is an inverse agonist at the angiotensin II type 1 receptor and because it reduces plasma angiotensin II levels.[23,27] Thus, overall, published data support the hypothesis that olmesartan should reduce rather than increase cardiovascular events. It is possible that mechanistic studies to assess potential harm have yet to be performed given that signals for potential harm have only been recently reported.

Similarly, no mechanisms to definitively explain the putative association between olmesartan and sprue-like enteropathy are known. Case reports indicate that symptoms appear months to years after olmesartan initiation.[28,29] Intestinal biopsies have revealed villous atrophy with mucosal inflammation and symptoms improve after drug discontinuation but not a gluten-free diet.[28,29] IgA transglutaminase antibodies are notably absent.[29] A cell-mediated or delayed hypersensitivity reaction, potentially associated with the human leukocyte antigen-DQ cell surface receptor type 2, has been proposed.[29]

Strengths of this study include the availability of a nationally representative, clinically rich data set; a relatively large sample size and long follow-up duration; a comparative effectiveness design in which olmesartan was compared directly against other ARBs; the use of advanced statistical techniques to adjust for potential confounders (including propensity score analysis); and conduction of extensive sensitivity analyses. Limitations include the retrospective, observational nature of the study design, the relatively short follow-up period (median 2.3 years was shorter than ROADMAP [median 3.2] and ORIENT [mean 3.2]), and the inability to adjust for additional potential confounders. The most important missing confounder was blood pressure, and we acknowledge that the observed differences in outcomes could have resulted from differences in blood pressure control. For example, in the overall cohort, subjects with losartan notably had less comorbidity at baseline, and the inability to adjust

Table 3. Subgroup Analyses in High-Risk Subjects Comparing Olmesartan Users With Users of All Other Angiotensin Receptor Blockers

	History of Cardiovascular Disease (n=8755)				CKD (GFR<60 mL/min; n=4575)			
Outcome	Adjusted HR (95% CI)	*P* Value	Time at Risk (Person -Years)	Events, n (%)	Adjusted HR (95% CI)	*P* Value	Time at Risk (Person-Years)	Events, n (%)
All-cause hospitalization or mortality	1.11 (0.99–1.24)	0.08	2008	363 (4)	1.21 (1.04–1.41)	0.02	1131	208 (5)
All-cause mortality	1.09 (0.59–2.03)	0.78	2462	12 (0)	0.88 (0.40–1.97)	0.76	1364	7 (0)
All-cause hospitalization	1.12 (0.99–1.25)	0.06	2008	362 (4)	1.23 (1.05–1.43)	0.009	1131	208 (5)
CV disease–related hospitalization	1.19 (0.98–1.46)	0.09	2335	115 (1)	1.30 (0.96–1.76)	0.09	1322	52 (1)
GI disease–related hospitalization	1.10 (0.87–1.37)	0.46	2348	91 (1)	1.27 (0.94–1.70)	0.12	1303	58 (1)
Noninfective enteritis and colitis-related admissions	1.13 (0.69–1.85)	0.62	2451	7 (0)	1.38 (0.79–2.42)	0.26	1351	9 (0)

CI indicates confidence interval; CV, cardiovascular; CKD, chronic kidney disease; GI, gastrointestinal; GFR, glomerular filtration rate; and HR, hazard regression.

for residual confounding may explain why there was a trend toward a lower hazard for the primary end point in olmesartan users in the overall group, yet risk was increased in the high-risk subgroups. Thus, it is important to emphasize that this type of study design provides associative and not causal evidence. In addition, all included subjects were middle aged Americans with commercial health insurance, which should be borne in mind when generalizing the results beyond this population. In particular, despite having cardiovascular risk factors or pre-existing disease, our study population had a crude death rate of only 392 per 100 000, which is lower than the 2010 crude death rate for all US adults aged 50 to 54 years (491.7 per 100 000)[30] and indicates that the study population was relatively healthy and well treated. Finally, we did not have information on cause-specific mortality and could not directly evaluate the association between olmesartan use and cardiovascular mortality.

Perspectives

Olmesartan is a commonly prescribed antihypertensive drug, and recent evidence linking this agent to an increased risk of cardiovascular mortality and sprue-like enteropathy mandates the need for further study. Analyses of large-scale clinical registry data serve as a useful and important complement to randomized controlled trial data in terms of assessing drug-related harm. In the present analysis, although there was a suggestion that patients with CKD may be at higher risk of all-cause mortality or hospitalization, findings that would be consistent with the results of the ROADMAP study,[3,4] our findings are not sufficiently robust or consistent to support the conclusion that olmesartan increases risk in patients with diabetes mellitus. About the subgroup of patients with CKD, given the results of ROADMAP and ORIENT and given our findings, we recommend that olmesartan use be used with caution in this patient population until further mechanistic, epidemiological, and interventional studies to clarify the effect of this drug on clinically important end points have been performed. We also recommend that further postmarketing surveillance of this agent be performed to assess risk in a more comprehensive fashion in different study samples and populations. This should take the form of additional analyses of clinical registries as well as a meta-analysis of individual patient-level data from previously published and soon-to-be-published randomized controlled trials.

Acknowledgments

R. Padwal originated the study idea and all authors contributed to the conception and design, the analysis, and interpretation of data. D.T. Eurich and M. Lin had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. R. Padwal and D.T. Eurich wrote the initial manuscript draft, all authors revised it critically for important intellectual content, and all authors provided final approval of the version to be published. We would also like to acknowledge Betsey Jackson at Health Data Services Corporation (www.hdscorp.biz), PO Box 53, Carlisle, MA 01741 for providing independent database acquisition services.

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Disclosures

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Novelty and Significance

What Is New?

- Olmesartan has been linked to an increased risk of cardiovascular mortality in patients with diabetes mellitus.
- We conducted a retrospective analysis of >45 000 subjects using a nationwide US-integrated insurance and laboratory claims database.
- In a risk-adjusted analysis that included propensity scores, no increased risk of all-cause mortality or hospitalization was found in our overall cohort although risk may be increased in patients with chronic kidney disease.

What Is Relevant?

- Olmesartan is commonly prescribed.
- To our knowledge, this is the first large comparative effectiveness study involving olmesartan in patients with diabetes mellitus.

Summary

We found no robust signal for harm and no compelling reason to avoid the drug except, perhaps, in patients with chronic kidney disease. Further study is required, especially in diabetics with chronic kidney disease.

Supplementary Methods

Dose-Response Sensitivity Analysis

A dose-response sensitivity analysis was also performed in which we used a standard (i.e., non-time dependent) Cox model to examine the association between tertiles of the average daily dose prescribed (low/medium/high) and the primary outcome in olmesartan users only. Subjects with the lowest level of exposure served as the reference group. Model covariates were identical to those used in the primary analysis except the propensity score adjusted for the propensity to receive a medium or high dose of olmesartan (compared to a low dose). To account for changes in dose over time, average daily dose was calculated by dividing the total dose prescribed over the follow-up period by the total drug exposure time. To calculate follow-up time, each subject was considered exposed to the drug until an event occurred (death or hospitalization), their insurance coverage was terminated or they discontinued therapy. If insurance coverage was terminated or treatment was discontinued, subjects were censored, with a censoring date of 60 days after the date on which their last prescription had ended. We also performed the same dose-response analysis for losartan and valsartan as a further sensitivity analysis. We did this to determine whether or not findings of the olmesartan dose-response analysis were similar for another ARB or specific to olmesartan alone.

Individual ARB Analysis

As a further sensitivity analysis, we performed an individual ARB analysis by dividing the primary cohort into separate ARB groups [olmesartan, losartan, valsartan, telmisartan and all others (candesartan, eprosartan and irbesartan)] and repeated the primary endpoint analysis (models adjusted as described above) to determine if olmesartan was associated with the highest risk of all-cause hospital admission or death. Olmesartan was used as the base comparator in this analysis, which was performed in the overall cohort and in the subgroups with pre-existing cardiovascular disease and chronic kidney disease. Subjects switching ARB agents were censored at the time the switch occurred.

Table S1. Sensitivity analysis examining the dose-response relationship within users of olmesartan, losartan and valsartan.

Group	Dose Tertiles	Medium Dose vs. Low Dose aHR (95% CI)	High Dose vs. Low Dose aHR (95% CI)
Olmesartan (n=10370)			
Overall cohort	Low: <18.7 mg Medium: 18.7-29.8 mg High: ≥29.9 mg	1.18 (1.04-1.34)	1.20 (1.05-1.37)
Cardiovascular disease	Low: <18.6 mg Medium: 18.6-30.1 mg High: ≥30.2 mg	1.62 (1.21-2.17)	1.40 (1.03-1.90)
Chronic kidney disease	Low: <19.9 mg Medium: 19.9-32.3 mg High: ≥32.4 mg	0.77 (0.51-1.14)	1.44 (0.99-2.10)
Losartan Sensitivity Analysis (n=8656)			
Overall cohort	Low: <37.4 mg Medium: 37.4-60.8 mg High: ≥60.8 mg	1.06 (0.96-1.19)	0.86 (0.77-0.97)

Cardiovascular disease	Low: <35.1 mg Medium: 35.1-56.2 mg High: ≥56.3 mg	1.14 (0.95-1.36)	0.86 (0.71-1.04)
Chronic kidney disease	Low: <36.6 mg Medium: 36.7-60.7 mg High: ≥60.8 mg	1.25 (0.96-1.63)	1.10 (0.83-1.47)
Valsartan Sensitivity Analysis (n=16004)			
Overall cohort	Low: <79.7 mg Medium: 79.8-143.1 mg High: ≥143.1 mg	1.24 (1.12-1.37)	1.43 (1.30-1.58)
Cardiovascular disease	Low: <78.33 mg Medium: 78.34-139.94 mg High: ≥140.0 mg	1.58 (1.32-1.89)	1.63 (1.36-1.94)
Chronic kidney disease	Low: <80.55 mg Medium: 80.57-147.42 mg High: ≥147.51 mg	0.71 (0.55-0.91)	1.06 (0.84-1.33)

aHR=adjusted hazard ratio; CI=confidence interval

Table S2. Sensitivity analysis comparing all-cause hospitalization or mortality in olmesartan users versus different ARBs

Agent (compared to olmesartan)	Overall Cohort (n=45185) HR (95% CI)	Cardiovascular Disease Subgroup (n=8755) HR (95% CI)	Chronic Kidney Disease Subgroup (n=4575) HR (95% CI)
Losartan (n=8656)	1.01 (0.94-1.08)	1.22 (1.07-1.40)	1.08 (0.90-1.30)
Valsartan (n=16004)	1.02 (0.96-1.09)	1.13 (0.99-1.28)	1.02 (0.86-1.20)
Telmesartan (n=3656)	0.94 (0.85-1.03)	1.09 (0.90-1.31)	0.87 (0.66-1.14)
All other ARBs (eprosartan, irbesartan, candesartan; n=6499)	1.00 (0.93-1.08)	1.03 (0.89-1.19)	0.79 (0.64-0.98)

Hazard ratios (HR) are relative to olmesartan (n=10370).

Exhibit P

2016 WL 4580145 (N.J.Super.L.) (Trial Order)
Superior Court of New Jersey, Law Division.
Atlantic County

Brandi CARL, Plaintiff,
v.
JOHNSON & JOHNSON, et al., Defendant.
Diana BALDERRAMA, Plaintiff,
v.
JOHNSON & JOHNSON, et al., Defendant.

Nos. ATL-L-6546-14, ATL-L-6540-14.
September 2, 2016.

Order

Mark C. Haggerty, Esquire, Michael R. Klatt, Esquire, Gene M. Williams, Esquire, Susan M. Sharko, Esquire, Julie Tersigni, Esquire, Lorna Dotro, Esquire, Hunter K. Ahem, Esquire, Kenneth J. Ferguson, Esquire, and Ann Thorton Field, Esquire.

Richard Golomb, Esquire, Ruben Honik, Esquire, Ted G. Meadows, Esquire, David B. Dearing, Esquire, Timothy W. Porter, Esquire, Michelle Parfitt, Esquire, and Paul R. D'Amato, Esquire.

Nelson C. Johnson, Judge.

*1 **THIS MATTER** having come before the court on Defendants' motions to bar expert testimony; and Defendants having filed companion motion(s) for summary judgment seeking I dismissal of Plaintiffs' Complaints in the event the motion(s) to bar testimony are granted; and Plaintiffs having filed cross motions to bar Defendants' expert testimony; and the court having conducted a plenary hearing on August 8, 9, 11, 12, 15, 16, and 19, 2016, at which time the court heard from Mark C. Haggerty, Esquire, Michael R. Klatt, Esquire, Gene M. Williams, Esquire, Susan M. Sharko, Esquire, Julie Tersigni, Esquire, Lorna Dotro, Esquire, Hunter K. Ahem, Esquire, Kenneth J. Ferguson, Esquire, and Ann Thorton Field, Esquire, on behalf of Defendants in support of their application; and Plaintiffs opposing this motion, Richard Golomb, Esquire, Ruben Honik, Esquire, Ted G. Meadows, Esquire, David B. Dearing, Esquire, Timothy W. Porter, Esquire, Michelle Parfitt, Esquire, and Paul R. D'Amato, Esquire, appearing; and the court having received expert testimony and oral argument of counsel conducted pursuant to *Evid. R.* 104 and 702, the standards articulated by our Supreme Court in *Kemp vs. The State of New Jersey* 174 *N.J.* 412 (2002), and for the reasons stated in the Opinion of even date herewith; and for good cause shown;

IT IS ON THIS 2nd DAY OF SEPTEMBER, 2016, **ORDERED** as follows:
1. Defendants' motion to bar the testimony of Dr. Graham A. Colditz is hereby GRANTED.

2. Defendants' motion to bar the testimony of Dr. Daniel W. Cramer is hereby GRANTED.

3. As a consequence of the aforesaid rulings, Defendants' motion for summary judgment as to Plaintiff, Brandi Carl, is hereby GRANTED. Plaintiff, Carl's Complaint is dismissed with prejudice.

4. As a consequence of the aforesaid rulings, Defendants' motion for summary judgment as to Plaintiff, Diana Balderrama, is hereby GRANTED. Plaintiff, Balderrama's Complaint is dismissed with prejudice.

5. As a consequence of the aforesaid rulings, Defendants' motions to bar testimony of other expert witnesses are deemed MOOT.

6. As a consequence of the aforesaid rulings, Plaintiffs' cross-motions to bar Defendants' experts are deemed MOOT.

<<signature>>

NELSON C. JOHNSON, JSC

I. POSTURE OF ISSUES BEFORE THE COURT

This matter is before the court on the motion of the Defendants, Johnson & Johnson and Imerys Talc America, Inc. (hereinafter referred to collectively as "Defendants") seeking relief against Brandi Carl and Diana Balderrama (hereinafter the "Plaintiffs"), both of whom brought claims alleging that a talc-based product manufactured by Defendants has caused each of them to develop ovarian cancer.

These two lawsuits were filed in the Superior Court of New Jersey, Atlantic County; the *Carl* matter on November 17, 2014 and the *Balderamma* matter on November 25, 2014. Pursuant to *R.* 4:38A, on October 20, 2015, the Supreme Court designated this litigation as a Multi-County Litigation (MCL), to receive centralized management by this court. The court is confident that, in these matters, every avenue of legal and scientific research has been explored by capable legal counsel and learned scientists, and that the litigants' interests have been well represented.

***2** Presently before the court is a challenge brought by Defendants to Plaintiffs' contention that the use of talc-based products caused them to develop ovarian cancer; said challenge was brought by motions to bar testimony of each of Plaintiffs' several expert witnesses. [NOTE: Defendants have filed companion motion(s) for summary judgment seeking dismissal of Plaintiffs' Complaints in the event the motion(s) to bar testimony are granted.] Defendants' challenge to Plaintiffs' experts was heard, and expert testimony, together with legal briefs and oral argument of counsel, were received by the court at a plenary hearing conducted pursuant to the standards articulated by the Supreme Court in *Kemp v. State of New Jersey*, 174 *N.J.* 412 (2002), (hereinafter a "*Kemp* Hearing") as required by *Evid. R.* 104 and consistent with *Evid. R.* 702. The court conducted said hearing on August 8, 9, 11, 12, 15, 16, and 19, 2016.

Defendants argue that Plaintiffs' hypotheses as to both general and specific causation are flawed; that there is no reliable scientific evidence to support Plaintiffs' contentions; and that accordingly, Plaintiffs' experts must be barred from testifying at trial. In reply, Plaintiffs argue that their experts are qualified by education, training, and experience and that their opinions are reliable because they are based on a sound scientific methodology, involving the type of information relied upon by experts in their field.

Thus, in evaluating the totality of the evidence presented by Plaintiffs, the question before the court may be stated as follows; Have Plaintiffs shown that their experts' theories of causation are sufficiently reliable as being based on a sound, adequately-founded scientific methodology, *to wit*, that they are based upon methods upon which experts in their field would reasonably rely in forming their own (possibly different) opinions about the cause(s) of each of Plaintiffs' ovarian cancers?

Courts are experts in the law, not science. This court's review "is as broad as the breadth of the proffer and the challenges thereto that the parties present." *Hisenaj v. Kuehner*, 194 *N.J.* 6, 19 (2008). Accordingly, this court's role is that of a "gatekeeper" who - based upon the proofs presented by the parties – must assess whether or not the hypotheses of causation advanced by Plaintiffs' experts are sufficiently reliable to be presented to a jury.

II. SCIENTIFIC STUDIES

Prior to receipt of testimony from the parties' experts, the court solicited from counsel the submission of all reports, abstracts, epidemiology studies, and peer-reviewed articles ("treatises" or "scientific literature") that were relied upon by the witnesses in formulating their opinions. That process began several months prior to the *Kemp* Hearing. As a result, approximately 100 treatises relating to talc, cancer, and miscellaneous related scientific issues were reviewed by the court both prior to and during the hearing. The court is grateful to counsel for these submissions; they were invaluable in preparing for the hearing and analyzing the evidence presented. [NOTE: Accompanying this ruling are Appendices A thru E which catalogue a portion of the peer-reviewed articles discussed at the hearing, together with public pronouncements by agencies possessing authoritative knowledge on cancer.]

Of particular value to the court in making its analysis is *The Reference Manual on Scientific Evidence* (3rd Edition, hereinafter, "the *Reference Manual")* issued by the Federal Judicial Center and the National Research Council of the National Academies. The *Reference Manual* is an invaluable tool. Because it is indicative of what the scientific community deems to be reasonable, the *Reference Manual* provides excellent guidance to trial judges in sifting through and prioritizing the information generated at a *Kemp* Hearing. At such a hearing, a court is asked to assess whether the experts in the field would reasonably rely on methods and data as Plaintiffs' experts have done in this case. Through the *Reference Manual*, the scientific community "speaks" to trial courts, and advises as to what may be considered to be reasonable, from an informed and objective perspective.

III. INITIAL FINDINGS RE: EXPERT WITNESSES

***3** Based upon consideration of the experts' written submissions and a careful review of all witnesses' testimony, together with the court's reading of the learned scientific treatises referenced herein, the court makes the following findings:

A. *Expert Witnesses*

The nine witnesses who testified at the *Kemp* Hearing are exceptionally learned and accomplished professionals; their credentials are impressive. No serious challenge was made to the qualifications of any witness. The court benefited greatly from their testimony. A brief profile of each witness follows:

Witnesses for Plaintiffs

(1) *Graham A. Colditz, M.D., MPH, DRPH, FAFPHM*: Dr. Colditz trained in Medicine at the University of Queensland, obtaining a M.B., B.S. degree. He trained in Epidemiology at Harvard School of Public Health, obtaining a Master of Public Health degree and subsequently a Doctorate. Dr. Colditz is the Niess-Gain Professor of Medicine at Washington University School of Medicine and the Associate Director, Prevention & Control, at the Alvin J. Siteman Cancer Center. He is the Chief of the Division of Public Health and Sciences in the Department of Surgery at Washington University School of Medicine. Dr. Colditz also serves as co-director of the Biostatistics Core for the Siteman Cancer Center. Dr. Colditz was presented on the issue of general causation of ovarian cancer.

(2) *Daniel W. Cramer, M.D., Sc.D.*: Dr. Cramer received his M.D. degree from the University of Colorado School of Medicine and a Doctor of Science degree in Epidemiology from the Harvard School of Public Health. Dr. Cramer is a Professor of Obstetrics, Gynecology and Reproductive Biology at Brigham and Women's Hospital, Harvard Medical School, and Professor of Epidemiology at the Harvard T.H. Chan School of Public Health. He heads the Research Division of the OB-GYN Epidemiology Center, doing research in the field of environmental and genetic risk factors for

a variety of obstetrical and gynecologic problems with a particular focus on ovarian cancer. Dr. Cramer was presented on the issues of both general and specific causation of ovarian cancer.

(3) *John J. Godleski, M.D.*; Dr. Godleski received his M.D. degree from the University of Pittsburgh School of Medicine. He is a Professor of Pathology at Harvard Medical School, Brigham and Women's Hospital, and a Professor of Environmental Health at Harvard TH Chan School of Public Health. Dr. Godleski has published more than 160 papers related to pulmonary/environmental pathology including a number using analytical electron microscopy. He currently leads the Particles Research Core in the Harvard-NIEHS Environmental Research Center and serves as Associate Director of the Harvard Clean Air Research Center supported by the US Environmental Protection Agency. Dr. Godleski was presented on the identification of particles, and on the issue of specific causation of ovarian cancer.

(4) *Curtis J. Omiencinski, Ph.D., ATS*: Dr. Omiencinski is an elected fellow and professor in the Academy of Toxicological Sciences and a Professor and the H. Thomas and Dorothy Willits Hallowell Chair in the Center for Molecular Toxicology & Carcinogenesis and the Department of Veterinary and Biomedical Sciences, College of Agricultural Sciences, at The Pennsylvania State University. He received his B.S. degree from the State University of New York at Albany and his Ph.D. degree in Pharmacology from the University of Washington's School of Medicine. He has authored more than 115 peer-reviewed papers and has published over 30 reviews, book chapters and other reports in the areas of pharmacology, molecular biology, toxicology, cancer research and genetics. His testimony was presented in connection with Plaintiffs' hypothesis of biologic causation of ovarian cancer.

***4** (5) *David C. Steinberg, MBA, FRAPS*: Mr. Steinberg owns a regulatory consulting firm for the cosmetic industry, specializing in the chemistry of cosmetic ingredients, preservatives and preservation, international and U.S. cosmetic regulations, and marketing of raw materials. He received his B.S. degree in Chemistry from Drexel University and an MBA Management degree from Pace University. He is a Fellow for the Regulatory Affairs Professionals Society.

Witnesses for Defendants

(1) *Lewis A. Chodosh, M.D., Ph.D.*: Dr. Chodosh is a physician and cancer researcher. He graduated *summa cum laude*, Phi Beta Kappa from Yale University with Distinction in Molecular Biophysics and Biochemistry. He received his M.D. degree from Harvard Medical School, graduating *magna cum laude* and his Ph.D. degree in Biochemistry from the Massachusetts Institute of Technology. Dr. Chodosh currently serves as Chairman of the Department of Cancer Biology and is a Professor in the Department of Cancer Biology and in the Department of Medicine in the Division of Endocrinology, Diabetes and Metabolism at the University of Pennsylvania School of Medicine. He also serves as Associate Director for Basic Science in the Abramson Cancer Center at the University of Pennsylvania, as well as the Director of Cancer Genetics at the Abramson Family Cancer Research Institute. Dr. Chodosh testified as to the diverse means by which cancer(s) develop in the human body and challenged the fundamental bases of Plaintiffs' biological hypothesis and contentions regarding specific causation.

(2) *Mary J. Cunningham, M.D.*: Dr. Cunningham is a board-certified gynecologic oncologist with GynOncology of Central New York in Syracuse, New York. She received her M.D. degree from Northwestern University Medical School. Dr. Cunningham serves as a Professor in the Department of Obstetrics and Gynecology and Director of the Division of Gynecologic Oncology at the State University of New York Upstate Medical University. She is a member of the American Congress of Obstetricians and Gynecologists and the Society of Gynecologic Oncology and the Principal Investigator for with the NRG Oncology cooperative trial group. Dr. Cunningham was presented in opposition to the testimony of Dr. Colditz and Dr. Cramer.

(3) *Elaine F. Schumacher*: Ms. Schumacher is a Senior Research Scientist and Analytical Microscopist with McCrone Associates, Inc. of Westmont, Illinois. She received her B.S. degree in Chemistry from Elmhurst College. Ms. Schumacher is a member of Microscopy Society of America, Midwest Microscopy and Microanalysis Society, Microanalysis Society

and American Chemical Society. In addition, she has authored several publications on the application of microscopy. Ms. Schumacher was presented in opposition to the testimony of Dr. Godleski.

(4) *Douglas L. Weed, M.D., M.P.H., Ph.D.*: Dr. Weed serves as a member of the Ethics Committee of the American College of Epidemiology. He received his B.S. and M.D. degrees from Ohio State University and his Ph.D. and M.P.H in Epidemiology degree from the University of North Carolina at Chapel Hill. Dr. Weed has 25 years of service at the National Cancer Institute ("NCI") and serves as a Visiting Professor at numerous universities. He is the Review Editor of the Journal of the NCI and a peer reviewer for many medical journals in the field of epidemiology. Dr. Weed has authored more than 30 peer-reviewed papers on causation methodology and systematic reviews, as well as meta-analyses of cancer epidemiology studies. Dr. Weed was presented in opposition to the testimony of Dr. Colditz and Dr. Cramer.

IV. CASE LAW PERTINENT TO THE COURT'S ANALYSIS

*5 As confirmed by the case law cited hereinafter, New Jersey's courts recognize that litigants claiming that they were harmed by the use of a product may never recover if they must await general acceptance by the scientific community of a reasonable, but not as yet certain, theory of causation linking the harm claimed to the product ingested. Because of our courts' concern that - despite compelling indicators linking a product to the harm - plaintiffs may never recover for their injuries, there are situations in which a theory of causation that has not yet reached general acceptance in the scientific community may still be found sufficiently reliable to support submission of such a claim to a jury.

In his learned essay first published in the *New Jersey Law Journal* on May 5th and 12th of 1988 (*see* 121 *N.J.L.J.* Index Page 882, *et seq.*), Justice Handler noted that "...there are many new classes of litigation, such as those involving exposure to toxic contaminants, asbestos and carcinogens, that pose complicated and novel problems." Justice Handler noted the "warfare" in our courtrooms is oftentimes resolved by the testimony of experts from diverse fields of knowledge:
The point is that there is no difference in the treatment of testimony of social scientists and psychologists, on the one hand, and chemists or biologists, on the other. Differences in acceptability have more to do with expanding frontiers of scientific knowledge.

121 *N.J.L.J.* Index at 883.

Until the final decade of the 20th Century, the time-honored test for the admissibility of expert testimony based upon a body of knowledge peculiar to a field of scientific study was that it had to be generally accepted or had been accepted by at least a substantial minority of the scientific community. *See Frye v. United States*, 54 *App. D.C.* 46 (D.C. Cir. 1923). In *Rubanick v. Witco Chem. Corp.*, 125 *N.J.* 421, 432 (1991), our Supreme Court modified that test with regard to evidence proffered for use in toxic tort cases. The Court held that a less stringent test than the general acceptance test should apply with regard to "new or developing theories of causation in toxic-tort litigation." *Id.* at 432. In writing for the Court, Justice Handler spoke of a methodology based test, that is, if the methodology by which the expert reached a conclusion is sound, the conclusion may be introduced into evidence. *Id.* at 438-40.

Pursuant to *Rubanick*, the key to reliability is the determination that the expert's opinion is based on a "sound, adequately-founded scientific methodology involving data and information of the type reasonably relied on by experts in the scientific field." *Id.* at 449. In order to be *valid methodology* (*viz.*, accepted by others in the scientific community), the expert's opinions must be supported by "prolonged, controlled, consistent, and validated experience." *Id.* at 436.

As this court understands *Rubanick*, in determining whether a scientific methodology is valid, trial courts must consider whether other scientists in the field use similar methodologies in forming their opinions and also should consider other factors that are normally relied upon by medical professionals. The appropriate inquiry is not whether the court thinks

that the expert's reliance on the underlying data was reasonable, but rather whether comparable experts in the field would actually rely on that information. With regard to evaluating the testimony of knowledgeable experts in order to determine the acceptability of a theory, the *Rubanick* Court cautioned trial courts to attend to "the hired gun phenomenon," *i.e.*, that an expert can be found to testify to the truth of almost any factual theory or to disagree with almost any theory and to discount the research of others. *Rubanick, supra* at 453 (citations omitted).

***6** Following *Rubanick*, in *Landrigan v. Celotex Corp.*, 127 *N.J.* 404 (1992), *Caterinicchio v. Pittsburgh Corning Corp.*, 127 *N.J.* 428 (1992), and *Dafler v. Raymark Industries, Inc.*, 259 *N.J. Super.* 17, 36 (App. Div. 1992), *aff'd. o.b.*, 132 *N.J.* 96 (1993), the Court held that experts relying on epidemiological studies could provide sufficient reliable evidence for the causes of diseases in specific individuals to present the issue of causation to juries. *Landrigan* and *Caterinicchio* involved the relationship of asbestos to colon cancer; *Dafler* addressed the relationship of cigarette smoking and asbestos to lung cancer.

In *Landrigan*, an occupational asbestos exposure case, the trial court dismissed the case on the ground that there was a lack of medical evidence to establish asbestos exposure as the cause of the disease. The Appellate Division affirmed. The Supreme Court reversed and held that epidemiologists could help juries determine causation in toxic tort cases and rejected the proposition that epidemiological studies must show a relative risk factor of "2.0" before gaining acceptance by a court, *Landrigan, supra* at 419. (A discussion of epidemiology and relative risk begins at p. 12).

The Supreme Court in *Landrigan* ruled that a trial judge must consider all the scientific data, sources thereof, and the methodology by which an expert reaches a conclusion, "includ[ing] an evaluation of the validity both of the studies on which he relied and of his assumption that the decedent's asbestos exposure was like that of the members of the study populations." *Id.* at 420. Additionally, the Supreme Court advised that "to determine the admissibility of the witness's opinion, [a] court, without substituting its judgment for that of the expert, should examine each step in [the expert's] reasoning." *Id.* at 421.

During the *Kemp* Hearing in these proceedings the court invited counsel to research what other courts have done on a relative risk factor of less than "2.0" and to submit their findings. The briefs furnished and the case law cited were very helpful. In reviewing the case law submitted by counsel, it is apparent that most courts across the nation - federal and state alike - discourage a dogmatic insistence upon a showing of a relative risk factor of "2.0" to support general causation. This court shares that perspective.

One case, cited by both sides, provided valuable guidance, namely *Magistrini v. One Hour Martinizing Dry Cleaning*, 180 *F. Supp.* 2d 584 (D.N.J. 2002), *aff'd*, 68 F. Appx. 356 (3d Cir. N.J. 2003). The court in *Magistrini* noted "[a]s a general matter, the Rules of Evidence 'embody a strong and undeniable preference for admitting any evidence' that could potentially assist the trier of fact and Rule 702 is liberally interpreted by the district courts." *Id.* 595 (citations omitted), *New Jersey Evidence Rule* 702 is identical to the Federal Rule. That said, the court in *Magistrini* also cautioned, "[t]he Court's inquiry 'must be solely on principles and methodology, not on the conclusions that they generate.'" *Id.* (citing *Daubert v. Merrell Dow Pharms.*, 509 *U.S.* 579, 595 (1993)). In articulating the mental process of the "gatekeeper," the court in *Magistrini* cited the Supreme Court decision in *GE v. Joiner*, 522 *U.S.* 136 (1997), wherein Chief Justice Rehnquist advised trial judges:

> But conclusions and methodology are not entirely distinct from one another. Trained experts commonly extrapolate from existing data. But nothing in either *Daubert* or the Federal Rules of Evidence requires a district court to admit opinion evidence that is connected to existing data only by the *ipse dixit* of the expert. A court may conclude that there is simply too great an analytical gap between the data and the opinion proffered.

***7** *Id.* at 146.

A reading of the case law as to the weight attached to a relative risk factor of less than "2.0" shows that it is only one of the factors to be considered by the court. What must also be examined are the foundational sources of the expert's opinions. As discussed herein (see p. 17) in connection with the court's examination of the "Bradford Hill" criteria, although no single criterion is dispositive, research performed prior to litigation and peer-reviewed essays on the scientific issue at hand are the basic means by which to demonstrate reliability. Where neither exists, an expert witness is obligated to explain to the court how she/he proceeded in arriving at his/her conclusions by referencing some objective source(s), *e.g.*, a peer-reviewed article in a reputable medical/science journal, the public pronouncements of an agency with respected authority on the issue, or a learned treatise on the issue, in order to demonstrate that she/he has followed the scientific method at the standard maintained by some recognized minority of scientists in his/her area of science.

Accordingly, as this court understands New Jersey law and our Supreme Court's holding in *Landrigan*, the admissibility of expert testimony in toxic tort cases "depends on the expert's ability to explain pertinent scientific principles and to apply those principles to the formulation of his or her opinion. Thus, the key to admission of the opinion is the validity of the expert's reasoning and methodology." *Landrigan, supra* at 414. Nonetheless, the Supreme Court noted that, traditionally, "plaintiffs have established a connection between tortious conduct and personal injuries through the testimony of medical experts who testify that the defendant's specific conduct was the cause of the plaintiffs' injuries[,]" but that "[t]oxic torts, however, do not readily lend themselves to proof that is so particularized." *Id.* at 415. Accordingly, plaintiffs in toxic tort cases "may be compelled to resort to more general evidence, such as that provided by epidemiological studies." *Id.* This court is, of course, bound by the holding in *Landrigan* that "when an expert relies on such data as epidemiological studies, the trial court should review the studies, as well as other information proffered by the parties, to determine if they are of a kind on which such experts ordinarily rely." *Id.* at 417. (In the course of analyzing the issues raised herein, the court has carefully read every epidemiological study cited by the witnesses and legal counsel at the *Kemp* Hearing).

Ten years after *Landrigan*, in *Kemp* v. *State of New Jersey*, 174 *N.J.* 412, 430-32 (2002), the Supreme Court applied the *Rubanick* standard to a case involving an injury allegedly caused by vaccination, and implied its applicability to all tort cases in which a medical cause-effect relationship has not yet been confirmed by the scientific community but for which "compelling" evidence suggests that such a relationship does exist. In *Kemp*, the Supreme Court suggested that an *N.J.R.E.* 104 hearing is the preferred procedural practice in every case involving an expert's theory that has not yet achieved "general acceptance," finding that the trial court has an obligation, *sua sponte*, to conduct such a hearing and that the failure to do so is plain error.

***8** Accordingly, from this court's perspective, the inquiry at a *Kemp* Hearing must be "flexible." Its focus must be on principles and methodology and not necessarily on the conclusions/opinions that such scientific methodology may generate. The trial court's role is to determine whether the expert's opinion is derived from a sound and well-founded methodology. "There must merely be *some expert consensus* that the methodology and the underlying data are generally followed by experts in the field." *Rubanick, supra* at 450 (Emphasis added). Thus, at this *Kemp* Hearing, Plaintiffs' burden is to demonstrate that the methodologies used by their experts are consistent with valid scientific principles accepted in the scientific and medical communities.

Finally, the court is guided by the words of Justice Handler in *Rubanick, supra*, 125 *N.J.* 451, wherein he cautioned trial court judges that they must exercise restraint.

> We do not believe that in determining the soundness of the methodology the trial court should directly and independently determine as a matter of law that a controversial and complex scientific methodology is sound. The critical determination is whether comparable experts accept the soundness of the methodology, including the reasonableness of relying on this type of underlying data and information. *Great difficulties can arise when judges, assuming the role of scientist, attempt to assess the validity of a complex scientific methodology*, (Emphasis added).

V. "BUILDING BLOCKS" OF THE SCIENTIFIC METHOD RELEVANT TO TALC-BASED POWDER AND OVARIAN CANCER

A *Kemp* Hearing is the intersection of the scientific method and the rule of law. If our court system is to be respected by the scientific community, then we must respect the scientific process. Essentially, the scientific method is the systematic pursuit of knowledge. This pursuit consists of those principles and procedures involved in the recognition and formulation of a problem, the collection of data through observation and experimentation, and the articulation and testing of a hypothesis by which to resolve the problem, and hopefully gain new knowledge useful to society.

What follows are the "building blocks" of the scientific method which the court must consider in evaluating Plaintiffs' experts' methodologies in arriving at their conclusions and opinions, and whether the same are "reliable." The key is consistent adherence to the scientific method. In addressing the issues to be resolved, the court has endeavored to faithfully apply the principles and tools of science to the issues at hand.

A. Epidemiological Studies

The two primary types of observational studies relevant to these proceedings (*viz.*, epidemiology studies) are (1) cohort studies, and (2) case-control studies. Cohort studies compare the incidence of disease among individuals exposed to a substance with an unexposed group. Case-control studies examine the frequency of exposure in individuals who presently have the disease and compare them to a group of individuals who do not have the disease.

Epidemiologic studies provide "the primary generally accepted methodology for demonstrating a causal relation between a chemical compound and a set of symptoms or disease." See *Conde v. Velsicol Chem. Corp.*, 804 *F. Supp.* 972, 1025-26 (S.D. Ohio, 1992), *aff'd*, 295 *F.3d* 1194 (11th Cir. 2002). When a scientific rationale doesn't exist to explain logically the biological mechanism by which an agent causes a disease, courts may consider epidemiologic studies as an alternate means of proving general causation. According to the *Reference Manual*, at page 723-24, large epidemiological studies present some of the strongest medical/scientific evidence. The typical use of large population-based studies is in connection with "general causation." As noted in the *Reference Manual* at page 623, general causation is concerned with "whether an agent increases the incidence of disease in a group and not whether the agent caused any given individual's disease." Nonetheless, the *Reference Manual* at page 552 cautions trial judges that "it should be emphasized that *an association is not equivalent to causation.*" (Emphasis in the original text).

***9** Epidemiologic studies attempt to identify agents that are associated with an increased risk of disease. Thus, the first question an epidemiologist must ask is whether or not an association exists between exposure to a substance and a particular disease. An association between exposure to an agent and a disease exists when the two occur together more frequently than they would by mere chance. In that situation, the association is referred to as *significant.* "Statistically significant" means that the scientific community recognizes that the association between two or more variables is caused by something other than "random chance." Once a significant association is observed, the scientist undertaking the study must assess the *strength* of the association, plus whether the reason for the observed association is due to *bias, chance or a genuine effect.* A measure of the strength of an association in an epidemiological study can be expressed in terms of its "relative risk" (hereinafter "R/R"). R/R indicates the difference in the risk of contracting a disease in people exposed to a substance, as compared to those who are unexposed but are otherwise similar, in this case the American adult female population. Determining the R/R is important in understanding the results of a study because virtually every disease associated with a risk factor also occurs, at some rate, in the general population among study participants who are unexposed to the risk factor.

R/R is commonly calculated by dividing the risk of developing a disease observed in an exposed group by the risk observed in an unexposed, but otherwise similar, group. If the risks of the unexposed and exposed are the same, then the relative risk estimate (which mathematically is simply the former divided by the latter) is "1.0", also termed "null." The null value indicates that exposure is not associated with the disease in that study. Thus, an R/R of "1.0" means that the agent has no effect on the incidence of disease. Similarly, if the R/R estimate is "1.3," then risk appears to be 30% higher among the exposed compared to the non-exposed. When an R/R reaches "2.0," the risk has doubled, indicating that the risk is twice as high among the exposed group as compared to the unexposed group. As discussed in the *Reference Manual* at page 612, note 192, there exists "... considerable disagreement on whether a relative risk of 2.0 is required or merely a taking-off point for determining sufficiency ...".

In evaluating epidemiological studies, it is important to note that "[a]n association is not equivalent to causation. An association identified in an epidemiological study may or may not be causal. Assessing whether an association is *causal* requires an understanding of the strengths and weaknesses of the study's design and implementation, as well as a judgment about how the study findings fit with other scientific knowledge." *Reference Manual* at page 552-3. As cautioned by the *Reference Manual*, the closer the R/R is to the null (or the further it is from 2.0), the greater the concern for bias or confounding.

Generally, there are three reasons that a positive association may be observed: (a) bias (including confounding factors), (b) chance, and (c) real effect. Each must be evaluated to extract a valid message from the study. Evaluation of these factors measures the "internal validity" of an epidemiology study, *viz.*, the extent to which a particular study's findings are viable and sound. "Bias" in epidemiology is systematic error, which includes "confounding bias." The underlying impact of these biases is to make the two groups being compared different in more ways than just the variable being studied. Sources of bias must be considered in interpreting an epidemiological study because bias can produce an erroneous association. *Reference Manual* at pages 591-3.

The record of the *Kemp* Hearing conducted by the court is replete with testimony, argument, and legal briefs regarding the significance to be attached to various studies conducted by epidemiologists on the possible association of talc-based products and ovarian cancer. Each side cited numerous studies to support its position. Nevertheless, this court's review of the various studies is informed by the admonishment of the *Reference Manual* at page 576:

> Common sense leads one to believe that a large enough sample of individuals must be studied if the study is to identify a relationship between exposure to an agent and disease that truly exists. Common sense also suggests that by enlarging the sample size (the size of the study group), researchers can form a more accurate conclusion and reduce the chance of random error in their results...With large numbers, the outcome of test is less likely to be influenced by random error, and the researcher would have greater confidence in the inferences drawn from the data.

B. Laboratory Studies on Talc and Cancer

***10** To confirm a possible cause-and-effect relationship suggested by epidemiological studies, an exposure assessment can be conducted in order that the findings of those studies may be compared to the adverse health impacts predicted from exposure estimates and toxicological data from laboratory experiments.

Laboratory studies can be conducted using cells from animals or humans. Research involving a controlled environment, such as cell cultures in a test tube or in a petri dish, are called *in vitro* studies. Studies done on living organisms are called *in vivo* studies. There are many institutions around the world conducting laboratory studies focused upon the potentially causal relationship between various substances and cancer. Much can be learned from those studies.

Here, regarding Plaintiffs' claim of a specific causal relation between talc-based powder and ovarian cancer, laboratory studies can be performed on both human and animal cells to assess the impact of talc upon tissue and cells removed from both women and animals.

C. Cancer Biology and Research

The past generation has seen large strides made in understanding the pathways which cause human cancers. These "pathways" are essentially a molecular chain of events that cause human cancers. Scientists now have the ability to analyze many thousands of genes, and to study how a particular gene responds to various substances. This can be done in both human and animal cells, both *in vitro* and *in vivo*. In the process scientists can gain a better understanding of what triggers cancer. Thus, understanding how these pathways get turned on or turned off by the mutations in key genes is critical to understanding the rudimentary causes of cancer. As will be discussed hereinafter in connection with the testimony of Dr. Lewis Chodosh, there is a great deal to be learned from studying the biology of cancer. The biology of cancer and the research being done (and results from years past) are all relevant to any scientific inquiry into the alleged causal connection between talc-based powder and ovarian cancer.

D. Animal Studies

Another means by which to measure the toxicity of an agent in humans is through animal toxicology studies. The purpose of animal studies is not to predict what specific types of cancer a particular carcinogen might cause in humans, but rather to identify whether it can cause cancer at all. However, animal studies are of limited use in determining whether a particular substance causes a particular disease, or type of cancer, in humans. Generally, where both epidemiologic studies and animal toxicology are available, there is no universal rule for how to reconcile them. The scientific method dictates that careful assessment of the methodological validity and power of the epidemiologic evidence must be undertaken and the quality of the toxicological studies and the question of interspecies extrapolation and dose-response relationship must be also considered.

E. Agencies Which Study Cancer

Though cancer has plagued mankind throughout the history of civilization, it wasn't until the twentieth century that the U.S. Congress decided to take the lead in developing a permanent agency of government to encourage research into the causes and cures of cancer.
In 1937, Congress established the National Cancer Act of 1937 to provide additional support for cancer research – it was the first time Congress had appropriated funds toward a non-communicable disease. The Act established the National Cancer Institute ("NCI") as the federal government's primary agency to address research and training needs for the cause, diagnosis, and treatment of cancer. NCI's responsibilities included (in part):

***11** • Conducting, coordinating, and promoting research and studies relating to the cause, diagnosis, treatment, and prevention of cancer.

• Reviewing and approving grant applications to support promising cancer research.

• Collecting, analyzing, and disseminating the results of cancer research conducted in the United States and in other countries.

[The above can be found at: http://www.cancer.gov/about-nci-overview/history.]

In addition to the NCI, several other agencies and associations study and report to the public. As shown in Appendix E, those entities include: U.S. Food and Drug Administration, American Cancer Society, World Health Organization, International Agency Research on Cancer, and The American College of Obstetricians and Gynecologists. [NOTE: Each of these agencies has made public pronouncements which are inconsistent with, and/or unsupportive of Plaintiffs' claims that talc-based powder causes ovarian cancer.]

F. Bradford Hill Criteria

From the court's perspective, this "building block" is really the "mortar" for the scientific method. The Bradford Hill criteria should be acknowledged, either initially or by way of summary, in any discussion of the method(s) by which scientists seek new knowledge on a given scientific question. Because this court sees the criteria discussed below as "mortar" for building the conclusions in this analysis, it is the final item discussed.

In 1965, respected scientist and pioneer in medical statistics, Sir Austin Bradford Hill (1897-1991), made a speech before a group of colleagues wherein he attempted to articulate those essential benchmarks which the scientific community must consider in distinguishing between causal and non-causal explanations of observed associations. That speech is likely the most widely-published and quoted after-dinner speech delivered by a physician.

In determining whether an observed association between a chemical and a disease is causal (*i. e.*, general causation), Hill advised that scientists should be guided by various factors, which are often referred to as the "Hill criteria."

These factors include: (1) **strength** of association (*i.e.*, is the association strong and statistically significant?); (2) consistency of the relationship (*i.e.*, whether it has been repeatedly observed in other persons?) (3) **specificity** of association (*i.e.*, is there a particular association between the substance and the condition it purportedly causes?); (4) **temporality** (are the cause and effect bound in time, or as Hill states, "which is the cart and which is the horse?); (5) **biological gradient** (does the association reveal a dose-response curve?); (6) **plausibility** (*i.e.*, whether there exists a biologically plausible *mechanism* by which the agent *could* cause the disease?); (7) **coherence** (does cause-and-effect interpretation of the data conflict with the history and biology of the disease?); (8) **experiment** (is the frequency of the associated events affected by reducing the amount of the suspected substance?); (9) **analogy** (should science anticipate similar results from a consideration of alternative explanations?). Here, regarding talc-based products and ovarian cancer, though most of the factors come in for consideration to varying degrees; this is particularly true factors 1, 2, 5, and 6, [NOTE: When, as here, the R/R is significantly less than "2.0", factor #6 is essential]

***12** Finally, it should be noted that it is unlikely that Hill intended that scientists should be inflexibly bound to his criteria. There is little doubt in the scientific community that he encouraged that the seven identified considerations be applied flexibly. That said, a final portion of his speech is worthy of quoting verbatim.

> All scientific work is incomplete – whether it be observational or experimental. All scientific work is liable to be upset or modified by advancing knowledge. *That does not confer upon us a freedom to ignore the knowledge we already have*, or to postpone the action it appears to demand at a given time. (Emphasis added).

VI. PRELIMINARY OVERVIEW OF EXPERT TESTIMONY AND ANALYSIS OF THE TOTALITY OF THE EVIDENCE PRESENTED

This court is ever mindful of its role as a "gatekeeper" and the "great difficulties" that can arise for a trial judge in ruling on the admissibility of expert testimony. The analysis for determining what proofs may be presented to a jury must be in accordance with the standards expressed by our Supreme Court; that is the frame of reference by which the information

presented by counsel and the experts must be scrutinized. The court had the opportunity to observe closely the nine expert witnesses presented by the parties. Much was learned from each witness; nonetheless, a preliminary observation sets the foundation for all that follows.

Throughout these proceedings the court was disappointed in the scope of Plaintiffs' presentation; it almost appeared as if counsel wished the court to wear blinders. Plaintiffs' two principal witnesses on causation, Dr. Daniel Cramer and Dr. Graham Colditz, were generally dismissive of anything but epidemiological studies, and within that discipline of scientific investigation they confined their analyses to evidence derived only from small retrospective case-control studies. Both witnesses looked askance upon the three large cohort studies presented by Defendants. As confirmed by studies listed at Appendices A and B, the participants in the three large cohort studies totaled 191,090 while those case-control studies advanced by Plaintiffs' witnesses, and which were the ones utilized in the two meta-analyses performed by Langseth and Terry, total 18,384 participants. As these proceedings drew to a close, two words reverberated in the court's thinking: "narrow and shallow." It was almost as if counsel and the expert witnesses were saying, *Look at this, and forget everything else science has to teach as.*

The *Reference Manual* expressly cautions against a narrow and shallow examination of the science supporting Plaintiffs' contentions. "The critical difference between cohort studies and case-control studies is that cohort studies begin with exposed people and unexposed people, while case-control studies begin with individuals who are selected based on whether they have the disease or do not have the disease and their exposure to the agent in question is measured." (p. 557). Additionally, Section IV. B. of the *Reference Manual* warns of bias, particularly "information bias" of the participants. "In a case-control study, potential information bias is an important consideration because the researcher depends on information from the past to determine exposure and disease and their temporal relationship." (p. 585).

Equally troubling is Plaintiffs' failure to address meaningfully the other fields of scientific inquiry – or "building blocks" – in support of their assertion of general causation, *e.g.*, laboratory studies on talc, cancer biology, and animal studies. Most critical is their failure to provide a coherent explanation to support their hypothesis for biologic plausibility, which is #6 of the Hill criteria, to wit, "plausibility".

***13** Neither Dr. Cramer nor Dr. Colditz expressed much interest in explaining just how it is that talc-based powder supposedly causes cancer in the ovaries, or for that matter any part of the human anatomy. "Inflammation" was used almost as a talisman that supposedly explained everything the court needed to know. Stated in lay terms, Dr. Cramer's and Dr. Colditz's postulation, essentially, is as follows: *The talc flows upstream and lodges in the ovaries; it irritates cells in the ovaries, causes inflammation, which in turn causes immunosuppression, and the inescapable result is cancer.* Positing that premise (which the court does not), both witnesses ignore the fact that that Dr. Godleski conceded on cross examination that he did not observe inflammation in any of the tissue -of either Plaintiff – that he examined.
Q Doctor, you agree also that neither Mrs. Carl nor Mrs. Balderrama's treating pathologists noted any talc-related inflammatory reactions in their reports in these cases?

A That's correct.

(See generally the testimony of 8/9/16; see P129, L1 thru P130, L21).

A cornerstone of the "talc causes cancer" hypothesis is "inflammation," yet none was present in any of the tissue samples studied.

Incident to the meager width and depth of the investigation employed by Plaintiffs' experts in this litigation was the failure to address several questions arising from the proffered evidence. These questions illustrate the flaws in the methodology of Plaintiffs' experts,

1. Those epidemiological studies showing a potential link between talc-based powder and ovarian cancer repeatedly rank serous ovarian cancer as the most likely type of cancer that may result among talc users. Dr. Cramer confirmed that in his testimony; "...invasive serous cancer, [is] the type most commonly associated with talc use." (Testimony of 8/8/16; see P320, L19) Neither Plaintiff was diagnosed with this condition. *Why was there no testimony presented to address this obvious incongruity?*

2. Talc was purportedly found in tissue surgically removed from each of the Plaintiffs. It was argued by Plaintiffs and their experts that inflammation is the root cause of all cancers. Yet there is nothing in the records nor expert reports demonstrating that the tissue samples were inflamed. *Why was there no testimony presented to address this obvious question?*

3. Positing Plaintiffs' contention that talc particles travel naturally through the female anatomy, from the perineum to the ovaries, then, *a fortiori*, the potential for talc particles to lodge elsewhere along the reproductive tract and create similar conditions would be apparent. Yet the only portion of the reproductive tract in which talc has purportedly caused cancer is the ovaries. Nothing was presented showing an increase in the other gynecologic cancers such as vaginal cancer, cervical cancer, uterine cancer, or fallopian tube cancer, which is what one would reasonably expect. *Why was there no testimony presented to address this obvious conundrum?*

Summary of Dr. Chodosh's Testimony

As part of its preliminary overview of the expert testimony presented, the court is compelled to highlight the testimony of one witness in particular. Dr. Chodosh's testimony for Defendants was akin turning on the lights in a dark room. The failure of Plaintiffs' experts to articulate a plausible hypothesis for the biological mechanism by which talc purportedly causes ovarian cancer is a serious deficiency. After hearing Dr. Chodosh's testimony, it is apparent to the court that there was no articulation of a plausible hypothesis because it is unlikely that one can be made. Dr. Chodosh's testimony illustrates the huge hole in Plaintiffs' scientific methodology, namely, the failure to consider the biology of cancer. Dr. Chodosh's testimony and the scientific studies (see Appendix D) upon which he relies in formulating his opinions appear to support a reasonable hypothesis that talc does not cause cancer because it cannot cause cancer.

***14** What follows are the most significant conclusions from Dr. Chodosh's testimony, none of which were addressed by anything Plaintiffs' experts presented, nor diminished in their impact on cross-examination.
1. Talc is *inert.* "...talc does not change gene expression in ovarian cells. Treating ovarian cells with talc didn't change the expression." (Testimony of 8/19/16; see P71, L2 thru P77, L13).

2. Talc is an anti-cancer property because it inhibits the formation of blood cells, and it cannot cause mutations.

Q What do they show just in some --

A In a thumbnail, it basically shows that talc actually inhibits the formation of blood vessel growth.

Q Which is an anticancer property of talc?

A Yes, that would be an anticancer property.

(See generally the testimony of 8/19/16; see P33, L23 thru P34, L7 and P39, L10 thru P53, L8).
See also the study by N. Najmunnis, et al., *Talc mediates angiostasis in malignant pleural effusions via endostatin induction* at Appendix D wherein these scientists concluded: "In conclusion, talc alters the angiogenic balance in the pleural space

from a biologically active and angiogenic environmental to an angiostatis milieu. Functional improvement following talc poudrage in patients with malignant pleural effusions may, in part, reflect these alterations in the pleural space."

3. Talc induces cancer cells to apoptosis but not to normal cells. (Testimony of 8/19/16; see P41, L5 thru P45, L3 and P143, L18 thru P145, L7).

4. It's universally accepted that mutations in critical genes is the mechanism that causes cancer, and talc doesn't cause mutations. (Testimony of 8/19/16; see P52, L22 thru P56, L9).

5. "Inflammation" is an extremely complex issue and it is unclear whether chronic inflammation is sufficient to induce cancer in the absence of a carcinogen. (Testimony of 8/19/16; see P177, L11 thru P181, L10).

VII. FOOD and DRUG ADMINISTRATION LETTER ON TALC

Much was made by counsel for both sides in their questioning of witnesses during the several days of the *Kemp* Hearing with regard to a letter from the Food and Drug Administration (FDA), dated April 1, 2014, hereinafter "the FDA letter." The FDA letter was in reply to the "Citizen Petitions" filed by Samuel S. Epstein, M.D., of the University of Illinois, School of Public Health, on behalf of the "Cancer Prevention Coalition." Said petitions (dated November 17, 1994 and May 13, 2008) requested the FDA to require all cosmetic talc products to bear a warning label, Particularly, with regard to talcum powder, the Coalition requested a prominent warning reading as follows: "Frequent talc application in the female genital area is responsible for major risks of ovarian cancer."

The court perused the FDA's letter on multiple occasions. Depending upon one's perspective, the letter can be cited for a great deal of importance, or, it might be said that the letter provides very little new information of significance to the issues that must be addressed herein. This court's reading falls into the latter category

There was limited discussion of the FDA's statutory and regulatory authority during the *Kemp* Hearing. Yet, there is a need to place the letter and the FDA's role into proper context. The pertinent regulation dealing with labeling of talcum powder or any other "cosmetic" product is set forth at Title 21 of the Federal Register. It states in pertinent part:
***15** §740.1 Establishment of warning statements.

(a) The label of a cosmetic product shall bear a warning statement whenever necessary or appropriate to prevent a health hazard that may be associated with the product.

(b) The Commissioner of Food and Drugs, either on his own initiative or on behalf of any interested person who has submitted a petition, may publish a proposal to establish or amend, under subpart B of this part, a regulation prescribing a warning for a cosmetic. Any such petition shall include an adequate factual basis to support the petition, shall be in the form set forth in part 10 of this chapter, and will be published for comment if it contains reasonable grounds for the proposed regulation.

Subpart "(a)" of Section 740.1 was discussed with one witness, and comments were made by counsel concerning the same. Yet there was no discussion by Plaintiffs' experts with regard to subpart "(b)." That subpart requires petitions such as those filed by Dr. Epstein and the Cancer Prevention Coalition to *include an adequate factual basis to support the petition.* Subpart "(b)" states that upon submission of an "adequate factual basis," the Commissioner of the FDA "either on his own initiative or on behalf of any interested person who has submitted a petition" has the authority to "publish a proposal to establish" a warning label for a "cosmetic product." That would include talcum powder. As noted by Deputy Director Steven M. Musser, Ph.D., the petitions were denied because they lacked sufficient "evidence of a

causal association between talc use in the perineal area and ovarian cancer." In denying the petitions, the "FDA found" and articulated six points which the agency concluded were supported by its review of "an expanded literature search."

Relevant to the court's analysis are findings #2 and #4 of the FDA letter. Finding #2 expressed concerns with biases in the design of studies and uncontrolled confounding. It also noted that "no single study has considered all the factors that potentially contribute to ovarian cancer". Finding #4 states in relevant part, "[a] cogent biological mechanism by which talc might lead to ovarian cancer is lacking..." Nothing was presented by Plaintiffs' expert with regard to these two critical findings of the FDA.

The FDA letter is essentially an acknowledgement of the status quo, based upon its own "expanded literature search." In short, the real rationale that can be drawn from the FDA letter is that if there existed sufficient evidence linking talc causally to ovarian cancer, *viz., an adequate factual basis to support* such a postulation, the FDA has the resources and regulatory authority to mandate a warning label for talcum powder.

VIII. DEFICIENCIES IN DR. COLDITZ'S METHODOLOGY

Dr. Graham Colditz is a brilliant scientist and a dazzling witness. His vocal inflection, cadence, and adroit use of histrionics are extremely effective. Dr. Colditz's reputation for his breadth of knowledge about cancer and the esteem in which he is held by his peers is well deserved. Yet, at times, it seemed that issues raised in these proceedings, and the questions posed to him, were a bit mundane for a scientist of his caliber.

***16** At page 10 of his report of July 31, 2015, Dr. Colditz discusses "biologic plausibility." His discussion of the subject entails fewer than 75 words. He cites a total of four peer-reviewed articles in arriving at his opinion: "Thus it is established that talc can travel to the ovary, it causes an inflammatory response, and this mechanism is consistent with the increase of ovarian cancer that is observed."

Scrutiny of the articles cited in Appendix C does not support his conclusion. What follows is a brief discussion of the aforesaid learned treatises referenced by Dr. Colditz.

Roberta B. Ness: This paper is limited to a review of existent epidemiologic literature in the English language on the risk and protective factors for ovarian cancer and "proposes a novel hypothesis that a common mechanism underlying this disease is inflammation." Though talc exposure is mentioned, along with other theories of what may cause ovarian cancer, this paper does not discuss the means by which *talc can travel to the ovary*, nor does it discus the means by which talc causes *an inflammatory response* in the cells of the ovaries.

Jack Cuzik: This paper is limited to use of aspirin and NSAIDs for cancer prevention. This treatise does not discuss the means by which *talc can travel to the ovary*, nor does it discuss the means by which talc causes *an inflammatory response* in the cells of the ovaries,

Britton Talbert: This paper is limited to the "multiple lines of evidence" which "suggest that ovarian cancer may be related to chronic inflammation." In short, "this pooled analysis supports the hypothesis that regular aspirin use reduces ovarian cancer risk." This treatise does not discuss the means by which *talc can travel to the ovary*, nor does it discuss the means by which talc causes *an inflammatory response* in the cells of the ovaries.

Britton Talbert: This paper is limited to a discussion of the pro-inflammatory mechanisms that may explain "the increased risk linked to more lifetime ovulations, endometriosis, and exposure to talc and asbestos, as well as the decreased risk with non-steroidal anti-inflammatory drugs." This treatise does not discuss the means by which *talc can travel to the ovary*, nor does it the means by which talc causes *an inflammatory response* in the cells of the ovaries.

Even the most generous reading of these four cited articles reveals that none of them proffers an articulation of a hypothesis – nor a means by which to test the same – setting forth a biologic mechanism by which talc-based powder may/can/possibly does cause ovarian cancer, Dr. Colditz's reliance upon these four treatises supports a finding by this court that he has failed to make a systematic review of the scientific literature and has ignored the rudiments of the scientific method in arriving at his conclusion that, "[t]hus it is established that talc can travel to the ovary, it causes an inflammatory response, and this mechanism is consistent with the increase of ovarian cancer that is observed."

Further, with regard to "biologic plausibility," the court recalls Dr. Colditz's answer to the questions posed from the bench on this issue. Those questions dealt with a hypothesis on biologic causation postulated by Dr. Cramer. The exchange between the court and Dr. Colditz reads as follows:
THE WITNESS: Yes, it is Dr. Cramer's study.

THE COURT: Then turn to page 355. I'm determined to get an answer to this question. I asked it yesterday, and I wasn't able to get an answer. 355. Look at the second column. And then let's go to the last long sentence. "We have also proposed that talc use during periods of ovulation may carry greater risk, based upon the hypothesis that ovarian surface epithelial disruption and repair accompanying ovulation might allow talc to become entrapped within the inclusions cysts that form with ovulation." First question is, explain that to me in laymen's terms.

*17 THE WITNESS: Wow. Ovulation.

THE COURT: A good scientist can do that. I'm sure you will. I understand ovulation.

THE WITNESS: You understand the ovulation. Right? That's --and so he's saying that with ovulation and then in that disrupted epithelium, the presence of talc can more likely get --

THE COURT: How? THE WITNESS: -- into a cell --

THE COURT: How? What's the cyst? What's an inclusion cyst?

THE WITNESS: Oh, so the -- this is the cyst that develops in an ovary that would have a talc particle in it as an inclusion cyst. So he's saying that with sort of the surface of the ovary has to repair each time it pops. And so there's --

THE COURT: That's a traumatic experience for that part of the body.

THE WITNESS: Yeah, right. And so there's inflammatory response.

THE COURT: Go ahead.

THE WITNESS: And so you got some macrophages and other things working to clean up and repair the epithelium. And if you've got the talc present at that time --

THE COURT: If you have it present at that time.

THE WITNESS: -- if you've ovulated, you've got higher likelihood is, I think, what he's trying to say.

THE COURT: And based upon your readings in preparation for your report, did you find any other peer-reviewed articles where Dr. Cramer discussed this hypothesis? And coupled with that, has anybody else discussed this hypothesis? Because if they do, I want to read it.

THE WITNESS: So obviously others have discussed the description of talc in ovary. The IARC and others describe inflammation and the carcinogenic process.

THE COURT: I've heard lots of testimony. But I'm talking about this hypothesis.

THE WITNESS: This actual --

THE COURT: I'm not asking you to defend this hypothesis.

THE WITNESS: No, no.

THE COURT: I'm asking you to tell me has anybody else discussed it so I can read it.

THE WITNESS: I can't think of this specific mechanism for getting in -- being described.

THE COURT: So you don't know of any other study where Dr. Cramer did or anybody else did?

THE WITNESS: To look at the inclusion cysts?

THE COURT: That's what it says.

THE WITNESS: No.

THE COURT: Okay. Then I still don't have an answer to my question.

THE WITNESS: Then you don't. It's a great question.

THE COURT: It doesn't mean it's a good question. It just means I don't have an answer to it.

THE WITNESS: This is why there's got to be continuing studies to understand this whole process better.

(Testimony of 8/16/16, P312, L13 thru P315, L19).

To summarize this court's understanding of Plaintiffs' inability to explain the biological mechanism for how talc causes cancer, Dr. Colditz noted candidly, "This is why there's got to be continuing studies to understand this whole process better."

Though there are additional deviations from the scientific method included in Dr. Colditz's report – namely, the manner in which he blithely passes over most of the Hill criteria – the most egregious may be his failure/refusal to discuss *strength* of association, and how the same supports general causation. Repeated use of the term "significant" with regard to the R/R adds something to the discussion, but not much. As noted above, this court cannot be inflexibly bound by a R/R of "2.0" nor are the Hill criteria. A review of Dr. Colditz's testimony – both on direct and cross-examination – fails to establish a single instance in which he states that any number less than "2.0" for the R/R equates to sufficient strength to find a causal relation. His testimony supports neither general nor specific causation, nor does it address the question of where or whether a "significant" relationship becomes "causal."

***18** Finally, Dr. Colditz's expert opinion is *ipse dixit* and has all the earmarks of a made-for-litigation presentation. We need look no further than his own past writings. *First*, in 2000 in his peer-reviewed article entitled, "Prospective Study of Talc and Ovarian Cancer," he concluded, "[o]ur results provide little support for any substantial association

between perineal talc use and ovarian cancer risk overall..." *Second*, in his "2004 Handbook of Cancer Risk Assessment and Prevention," he lists talc as a "factor under study" in lieu of a modifiable factor which increases the risk of ovarian cancer. *Third*, as of 2011, on the website of the Alvin J. Siteman Cancer Center of which he is the Associate Director, the consensus of the Siteman scientific panel – which included both Dr. Colditz and Dr. Cramer – concluded that it was not appropriate to list talc as a risk factor on the "Your Disease Risk" portion of the website.

There is no challenge to Dr. Colditz's qualifications, nor that his testimony is relevant. Yet from the court's perspective, there are significant gaps in his methodology and analysis. He has committed the very error which Hill warned scientists against, namely, that the results of their research "...does not confer upon us a freedom to ignore the knowledge we already have." Dr. Colditz has overlooked the knowledge to be learned from laboratory research regarding the biology of cancer.

Applying the standards established in *Rubanick, supra*, 125 *N.J.* at 449, and *Landrigan, supra*, 127 *N.J.* at 420-1, the court concludes that the significant deficiencies in Dr. Colditz's methodology and analysis herein described, render his opinions inadmissible in these proceedings, and that the Defendants' motion to bar the testimony of Dr. Colditz is hereby GRANTED.

IX. DEFICIENCIES IN DR. CRAMER'S METHODOLOGY

Dr. Cramer is a distinguished professional. His commitment to medical science generally, and to learning more about the potential health consequences to women from the frequent use of talcum powder in particular, have been unswerving throughout his career. Few people possess the knowledge he has acquired from case-control studies regarding the potential effects of talc *vis a vis* ovarian cancer. His passion for this subject is palpable and exemplary.

Dr. Cramer's study of this subject together with his examination and his analysis of the results of many case-control studies addressing the relationship between talc and ovarian cancer date back more than 30 years. In July, 1982 he published his initial peer-reviewed article on this subject entitled, "Ovarian Cancer and Talc: A Case-control Study." Over the past 34 years, Dr. Cramer has authored and co-authored numerous peer-reviewed articles on talc. He has also conducted several meta-analyses of other epidemiology reports. All those studies appear to demonstrate a consistent, albeit uniformly weak, association between talc and ovarian cancer.

Dr. Cramer is highly qualified and his testimony is relevant. Yet from the court's perspective, there is a large gap in his methodology. Dr. Cramer has totally ignored laboratory research regarding the biology of cancer and the ameliorative effects of talc on cancer. He has made the error that Hill expressly warned scientists against, viz., that the results of their research "...does not confer upon us a freedom to ignore the knowledge we already have."

As discussed above, the research and existing studies cited in the testimony of Dr. Chodosh dismantled the premise of Dr. Cramer's opinions on the causal association between talc-based products and ovarian cancer. Dr. Cramer's failure to address the opinions of Dr. Chodosh and the results of laboratory research on the ameliorative effects of talc on cancer highlights the serious flaws in his methodology.

For purposes of this *Kemp* Hearing, the court must consider whether Dr. Cramer's testimony is sufficiently reliable to be presented to a jury. Defendants attack his opinions on both *general* and *specific* causation.

***19** On the issue of *general causation*, Defendants attack the odds ratios (O/R) established in his report. Dr. Cramer notes that in general, his research — relying almost entirely upon case-control studies – confirms that there is an O/R of 1.29 between perineal talc use and ovarian cancer. As indicated in his report, Dr. Cramer performed a case-control study to generate his final conclusions. In both his report and in his testimony, Dr. Cramer opines that the causal association between ovarian cancer and the use of talc has been "significant" and consistent for 30 years. The O/R of 1.29 reported by

Dr. Cramer is admittedly "weak" and neither he nor any other witness explained when/how a "significant" association becomes causal?

A retrospective case-control study is commonplace in the field of epidemiology, but as noted by the *Reference Manual* at page 576 such studies are considered less reliable than a prospective cohort study. Yet, that is almost entirely where Dr. Cramer devotes his research. According to Dr. Cramer, there have been 19 peer-reviewed scientific articles addressing the talc and ovarian cancer association since 1982. More recently there have been three very large cohort studies whose number of participants dwarfs those of the case-controls studies. (See Appendix A). Undermining the reliability of his testimony, Dr. Cramer is rigidly dismissive of the knowledge to be gained from the much larger cohort studies. On cross-examination, when asked if he had performed a meta-analysis of the three large cohort studies, he tartly replied, "I have not done that. The defense is very capable of doing that themselves." (Testimony of 8/8/16; see P324, L1 thru L8. See also his testimony at P199, L24 thru P200, L5).

Most troubling to the court is the effort made by Dr. Cramer to use epidemiology to prove *specific* causation. As noted by the *Federal Manual* at page 553, trial judges are warned of the overreliance upon such studies, "[a] final caveat is that employing the results of group-based studies of risk to make a causal determination for an individual plaintiff is beyond the limits of epidemiology." And again, the *Federal Manual* cautions, "[e]pidemiology is concerned with the incidence of disease in populations, and epidemiologic studies do not address the question of the cause of an individual's disease. This question, often referred to as specific causation, is beyond the domain of the science of epidemiology." (p. 608). In short, Dr. Cramer's methodology appears to be litigation driven rather than objectively and scientifically grounded.

The court uses the phrase *made-for-litigation* methodology for a reason. In all his prior peer-reviewed articles, Dr. Cramer never once stated that he believes talc causes ovarian cancer; not in his articles of 1982, 1999, 2000 (with Gertig) and 2007 does he make such an assertion. In fact, in his study of 2007, he concluded, "[w]e are not claiming that a causal relationship between ovarian cancer and talc is proven for this case or in general." Yet now, after having never made such a claim, he asserts here not only general causation, but specific causation as to both Plaintiffs, and purports to do so by re-analyzing old studies and subjectively mingling the various risk factors for each Plaintiff in order to prove ovarian cancer *by the numbers.* This "methodology" is not one based upon "prolonged, controlled, consistent and validated experiences". *Rubanick* at 436.

A final issue which must be addressed with regard to specific causation is the detailing of a hypothetical etiology of the disease in question and how the alleged substance is the malefactor. In his study of 1999 (See Appendix B), Dr. Cramer – in passing – made a partial articulation of a hypothesis for the biological mechanism by which talc purportedly causes ovarian cancer. That partial articulation is set forth in a single sentence which reads:

> ***20** We have also proposed that talc use during periods of ovulations may carry greater risk, based on the hypothesis that ovarian surface epithelial disruption and repair accompanying ovulation might allow talc to become entrapped within the inclusion cysts that form with ovulation. (p. 355).

This is the closest Dr. Cramer has ever come to postulating a hypothesis for the causal link between talc and ovarian cancer. He does not allude to this hypothesis in either the Carl or the Balderamma reports. Nor was he asked about this hypothesis by counsel on direct-examination.

Instead of a plausible explication of a hypothesis setting forth the biological mechanism of the causal link between talc-based powder and ovarian cancer, what the court received was a *made-for-litigation* methodology, to wit, the subjective mingling of risk factors to advance the base-line relative risk for each of the Plaintiffs (as members of the U.S. population) from 1.29 to 1.75 (Carl) and 1.79 (Balderamma). The knowledge learned to date from epidemiology studies involving talc and ovarian cancer is insufficient to prove ovarian cancer *by the numbers.*

Each of the Plaintiffs had significant risk factors for ovarian cancer to which Dr. Cramer's testimony showed a stark indifference. Ms. Carl had the following risk factors: obesity, nulliparity, infertility, past use of an IUD, psychotropic medication, smoking, and exposure to hair dye. Ms. Balderamma had the following risk factors: obesity, nulliparity, irregular cycles, early menarche (age 11), polycystic ovarian syndrome, past use of an IUD, and a potential BRCA gene diagnosis.

Despite his failure to eliminate – or make an objective accounting of- those multiple risks, Dr. Cramer leaps to specific causation *by the numbers.* He is not concerned that he hasn't even attempted to postulate a plausible biological hypothesis for how talc causes ovarian cancer as urged by factor #6 of the Hill criteria. His opinions rely upon an incomplete/ irregular methodology unlike anything upon which his peers would rely, and appear to be grounded only in his instincts and personal predilections. In short, the mingling of various risk factors and the purported "synergy" between talc and other health conditions is highly speculative and does not conform to any methodology utilized in the scientific community.

Finally, Dr. Cramer and Plaintiffs' counsel would be better served to heed the wisdom contained in the FDA Letter of April 1, 2014. Finding #4 of "Epidemiology and Etiology Findings" reads in pertinent part: "A cogent biological mechanism by which talc might lead to ovarian cancer is lacking..." Hill criterion #6, to wit, **plausibility** (*i.e.*, whether there exists a biologically plausible *mechanism* by which the agent *could* cause the disease?) requires Plaintiffs' experts to articulate and support/defend a plausible *mechanism* by which talc *could* cause ovarian cancer. Their failure to do so is decisive in the court's analysis.

Applying the standards established in *Rubanick, supra*, 125 *N.J.* at 449, and *Landrigan, supra*, 127 *N.J.* at 420-1, the court concludes that the significant deficiencies in Dr. Cramer's methodology and analysis herein described, render his opinions inadmissible in these proceedings, and that the Defendants' motion to bar the testimony of Dr. Cramer is hereby GRANTED

X. RULING

***21** As is true of most adversarial proceedings, the written reports and testimony of Plaintiffs' experts are much like a patch-work quilt; individual pieces that when sewn together create a single blanket. If well sewn, the blanket covers the issues required to meet Plaintiffs' burden of proof. Positing, for the sake of discussion, that each piece of cloth is sound, the fragments cannot become a quilt without thread. Without a clearly stated, demonstrable hypothesis of specific causation, grounded in a reliable methodology, there is no thread and the pieces of cloth remain disparate.

Accepting, for the sake of discussion, that the case-control studies relied upon by Dr. Cramer — to the exclusion of cohort studies, laboratory studies, cancer biology and the pronouncements of those agencies that study cancer – convey an inference that there is some type of causal association between talc and ovarian cancer, it means nothing without a hypothesis of specific causation. No witness for Plaintiffs ventured to articulate just how it is that talc in the ovaries, or, what it is about talc in the ovaries, that sets off a chain of events which purportedly causes ovarian cancer. Uttering the term inflammation does not explain the etiology of ovarian cancer, nor can the manipulation of numbers serve as a hypothesis for specific causation. Absent the thread, there is no quilt.

As the proponent of the evidence on general and specific causation, "the plaintiff bears the burden of establishing admissibility." *Kemp, supra*, 174 *N.J.* at 429. As discussed, the testimony of Plaintiffs' experts suffers from multiple deficiencies, the most salient of which are the narrowness and shallowness of their scientific inquiries and the evidence upon which they rely. Their peers in the scientific community would not rely upon such limited information.

Ultimately the admissibility of these experts' opinions depends "on the trial court's assessment of both [their] qualifications and [their] methodology." *Landrigan, supra*, 127 *N.J.* at 422. "The key to the admission of the opinion

is the validity of the expert's reasoning and methodology." *Id.* at 414, Though both Plaintiffs' experts are eminently qualified, their areas of scientific inquiry, reasoning, and methodology are slanted away from objective science and towards advocacy. It is this court's conclusion that the opinions expressed by Plaintiffs' experts fail to demonstrate "that the data or information used were soundly and reliably generated and are of a type reasonably relied upon by comparable experts." *Rubanick, supra*, at 477.

For the reasons stated herein, the Defendants' motion to bar expert testimony and for entry of summary judgment as to both the Carl and Balderrama matters are hereby GRANTED.

With regard to the other expert witnesses of the Plaintiffs as well as Plaintiffs' cross-motions to bar the Defendants' experts, the Court will neither opine nor rule on the same. In light of the foregoing ruling, said petitions are of no practical significance and are deemed MOOT.

<<signature>>

NELSON C. JOHNSON, J.S.C.

Date of Decision: 9/2/16

Appendix not available.

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Exhibit Q

AP&T Alimentary Pharmacology and Therapeutics

Immunopathogenesis of olmesartan-associated enteropathy

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SUMMARY

Background
Olmesartan-associated enteropathy (OAE) is characterised by diarrhoea, nausea, vomiting, abdominal pain, weight loss and severe sprue-like enteropathy, all of which are resolved after discontinuation of olmesartan medoximil.

Aim
To determine the mechanistic similarities of OAE with coeliac sprue.

Methods
Duodenal biopsies were extracted from OAE patients before (n = 11) or after (n = 17) discontinuation of olmesartan medoxomil (on or off olmesartan medoxomil). There were seven 'on/off' paired samples. Formalin-fixed biopsies were stained for CD8, CD4, FoxP3, IL-15R and psmad 2/3. Caco2 cells (human colonic epithelial line) were treated with olmesartan medoxomil and stained for IL-15, IL-15R and ZO-1.

Results
In the 'on olmesartan medoxomil' duodenal biopsies, a significant increase in the numbers of CD8+ cells and the number of cells that are FoxP3+ (a regulatory T-cell marker) are present in the duodenum as compared to the duodenal biopsies from patients who discontinued olmesartan medoxomil. IL15R expression is also increased with olmesartan medoxomil use. Evaluation of the effect of olmesartan medoxomil upon Caco-2 cells demonstrated that IL15 expression is increased in response to olmesartan medoxomil treatment. Further, ZO-1, a tight junction protein, is disrupted in olmesartan medoxomil-treated Caco-2 cells.

Conclusions
Olmesartan-associated enteropathy shares many features with coeliac disease, including symptoms and immunopathogenic pathways, such as increased numbers of CD8+ cells and corresponding overexpression of IL15 by epithelial cells. Taken together, the treatment of epithelial cells with olmesartan medoxomil induces a response by intestinal epithelial cells that is similar to the innate effects of gluten upon the epithelium of coeliac patients.

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INTRODUCTION

Olmesartan-associated enteropathy (OAE) is an enteropathy associated with the use of olmesartan medoxomil that resolves with the discontinuation of the drug.[1] It is characterised by diarrhoea, nausea, vomiting, abdominal pain and weight loss. The occurrence of OAE in individuals who routinely use olmesartan medoxomil is currently unknown but is thought to be rare. At Mayo Clinic, 35 individuals have been diagnosed with OAE in the last 5 years, and a national study done in France identified 31 OAE patients.[2, 3] There have also been multiple case reports from around the world.[4–11] Although the frequency for OAE appears to be low, it is imperative to rapidly identify these individuals, because complications of OAE are severe, including renal failure, and suspension of the drug leads to resolution of symptoms and mucosal healing.[12, 13] Olmesartan is an angiotensin II receptor blocker (ARB) and is administered as a prodrug (olmesartan medoxomil).[14] Of these, only olmesartan medoxomil has been consistently reported to be associated with enteropathy.[1] Olmesartan differs from most other ARBs in the attachment of the medoxomil moiety. The one other ARB with medoxomil, azilsartan, was approved by the US FDA on 25 February 2011 and is not widely used. As yet, no associations between azilsartan and enteropathy have been reported. Histopathologically and clinically, OAE appears to have many similarities to coeliac disease and some similarities to autoimmune enteropathy.[1, 13, 15, 16]

To determine the mechanisms that occur in OAE, we did a set of analyses that were inspired by the marked similarities in both the clinical symptoms and histopathology shared between coeliac disease and OAE. As a starting point, we first took duodenal biopsies from the OAE patients before and/or after they discontinued their use of olmesartan medoxomil (on or off olmesartan medoxomil) and then characterised the cell types of the infiltrate.

MATERIALS AND METHODS

Diagnostic criteria for OAE

The following three criteria were used for diagnosis of OAE.[1]

(i). Chronic diarrhoea (>4 weeks) while taking olmesartan medoxomil.

(ii). An alternate cause for the enteropathy could not be established after a systematic diagnostic evaluation that included investigation for disorders associated with nonresponsive coeliac disease as previously reported by our group.

(iii). Clinical improvement after discontinuation of olmesartan medoxomil.

OAE patients

Duodenal biopsies from 26 OAE patients were used for the immunohistochemistry analyses. Of the 26 OAE patients, 11 were men and 15 were women. For the alternate diagnosis evaluation, the medical histories of 35 OAE patients were reviewed. For this, the ratio of males to females was again 0.7–1.0.[3]

Extraction of duodenal biopsies

For the diagnostic evaluation, a small bowel biopsy was done before withdrawal of the drug (on olmesartan) and one after discontinuing the use of olmesartan ('off olmesartan'). 'Off olmesartan' biopsies were defined as biopsies performed at least 30 days after the date of suspension of olmesartan with a mean of 3 months after date of suspension. Duodenal tissue was immediately placed into formalin and later embedded into paraffin. There were seven paired small bowel biopsy samples, with which a biopsy was taken while the patient was on olmesartan medoxomil (on) and another paired biopsy was taken after the same patient discontinued olmesartan medoxomil (off). There were an additional four 'on' samples and an additional 10 'off' samples that did not have a corresponding paired sample.

Immunohistochemistry

Immunohistochemical staining was done at the Mayo Pathology Resource Core Facility. Antibodies used were purified anti-CD8 (DAKO, Glostrup, Denmark), purified anti-CD4 (DAKO), purified anti-FoxP3 (Abcam, Cambridge, MA, USA), purified anti-granzymeB (DAKO), purified anti-IL-15R (Biorbyt, San Francisco, CA, USA) and purified anti-psmad2/3 (Santa Cruz Biotechnology, Dallas, TX, USA).

Treatment of Caco-2 cells with olmesartan medoxomil, olmesartan acid, diacetyl (medoxomil) Telmisartan and Losartan

Caco2 cells purchased from ATCC (American Type Culture Collection, Manassas, VA, USA) were treated with trypsin (0.05% Gibco of Life Technologies, Carlsbad, CA, USA) and then cultured in media for 7 days on tissue culture microscope slides (Nunc Lab-Tek II Chamber Slide System – Thermo Scientific, Waltham,

MA, USA). Media was EMEM (Eagle's Minimum Essential Medium-American Type Culture Collection) supplemented with 10% foetal bovine serum and penicillin/streptomycin. Olmesartan medoxomil (Benicar from Schering-Plough, Kenilworth, NJ, USA) was ground into fine particles and then resuspended into HCl (pH 6.8). Losartan (Thermo Fisher Scientific) was resuspended into phosphate-buffered saline (PBS). Olmesartan acid (Santa Cruz Biotechnology) was resuspended in DMSO (Dimethyl Sulfoxide-Sigma Aldrich), as well as telmisartan (Thermo Fisher Scientific). Diacetyl (Sigma-Aldrich, St. Louis, MO, USA), the three different ARBs and DMSO were then added to the Caco 2 cells at 30 μmol/L concentration in 2 mL of fresh media. Exposure times were between 30 min to 4 h and are described in each figure legend.

Immunofluorescent analysis

Caco-2 cells were fixed in 3.7% formaldehyde and stained with purified anti-ZO-1 (Invitrogen of Thermo Fisher Scientific, Grand Island, NY, USA), purified anti-IL-15 (BioRad, Hercules, CA, USA) or purified anti-IL-15R (BiOrbyt). The secondary antibody used was FITC conjugated anti-rabbit IgG (Jackson Immunoresearch, West Grove, PA, USA). Nuclei were stained using DAPI (4′,6-Diamidino-2-phenylindole; Sigma Aldrich). Final evaluation and image capture of the staining was done with a confocal laser microscope (LSM 780, Zeiss, Thornwood, NJ, USA) using Zen 2010 software. Immunofluorescence quantitation was done using ImageJ (1.48v) software, available from the National Institutes of Health (Bethesda, MD, USA).

Statistical analysis of immunohistochemistry

For the CD8, CD4, FoxP3, psmad 2/3 and IL15R staining, the average number of positive cells in a villous/crypt unit was calculated after counting the total number of positive cells for three villous/crypt units, wherein a villous/crypt unit of area is defined as the area of one villous and the area below that villous extending through the crypt layer. For the epithelial staining of IL15R, and the intracellular staining of psmad 2/3 in the lamina propria, an intensity score was calculated. For the psmad 2/3 staining, 0 was no staining, 1 and 2 were given for weak and intermediate intensity, and 3 was the highest intensity of staining. For the IL15R staining, 0 was given for no staining, 1 was intermediary intensity and 2 was the highest intensity of staining. The statistical significance of the differences in total number of positive cells of each staining in a villous/crypt unit between the on and off olmesartan groups was assessed by the Mann–Whitney rank-sum test. The on group consisted of 11 samples and the off group 17 samples for CD4, CD8, FoxP3 and psmad 2/3 staining. The differences in intensity scores between the two groups were assessed by the Mann–Whitney rank-sum test. Graphpad Prism6 (GraphPad Software, La Jolla, CA, USA) was used to conduct the analyses.

RESULTS

Distribution of CD8+ and CD4+ cells

Previously we had published that lymphocyte infiltration of the small bowel (duodenum) occurred in patients with OAE.[1] To determine the cellular composition of the lymphocytic infiltration, anti-CD8 (Figure 1a–c) and anti-CD4 (Figure 1d–f) staining was done on duodenal biopsies from OAE patients while they were on olmesartan medoxomil (Figure 1a,d) or off olmesartan medoxomil (Figure 1b,e). Results from the CD8 staining demonstrate a significant increase ($P < 0.05$) in the number of CD8+ cells in the duodenum of OAE patients on the drug as compared to off the drug (Figure 1c). This analysis was done on unpaired samples (11 on olmesartan medoxomil, 17 off olmesartan medoxomil). In contrast, the analysis of CD4+ cells did not reveal a significant difference ($P = 0.67$) in the number of CD4+ cells in the small intestine of OAE patients, comparing on and off olmesartan medoxomil (Figure 1f).

Distribution of Granzyme B+ cells

We next evaluated the expression of granzyme B to determine the potential presence of cytotoxic T lymphocytes (CTLs). Figure 2a shows greater numbers of granzyme B+ cells while on olmesartan medoxomil, as compared to off (Figure 2b), indicating that increased numbers of CTLs are present in the infiltrates of the duodenum of OAE patients. However, this was not statistically significant (Figure 2c) ($P = 0.1$ unpaired t-test using Welch's correction).

Distribution of FoxP3+ cells

To determine if the OAE patients have a deficiency in their intestinal regulatory T cells, we next addressed whether olmesartan medoxomil treatment decreased the number of FoxP3+ cells. Figure 3a (on olmesartan medoxomil) and b (off olmesartan medoxomil) demonstrate that the patients did have a significant increase in the number of FoxP3+ cells (Figure 3c) ($P < 0.05$

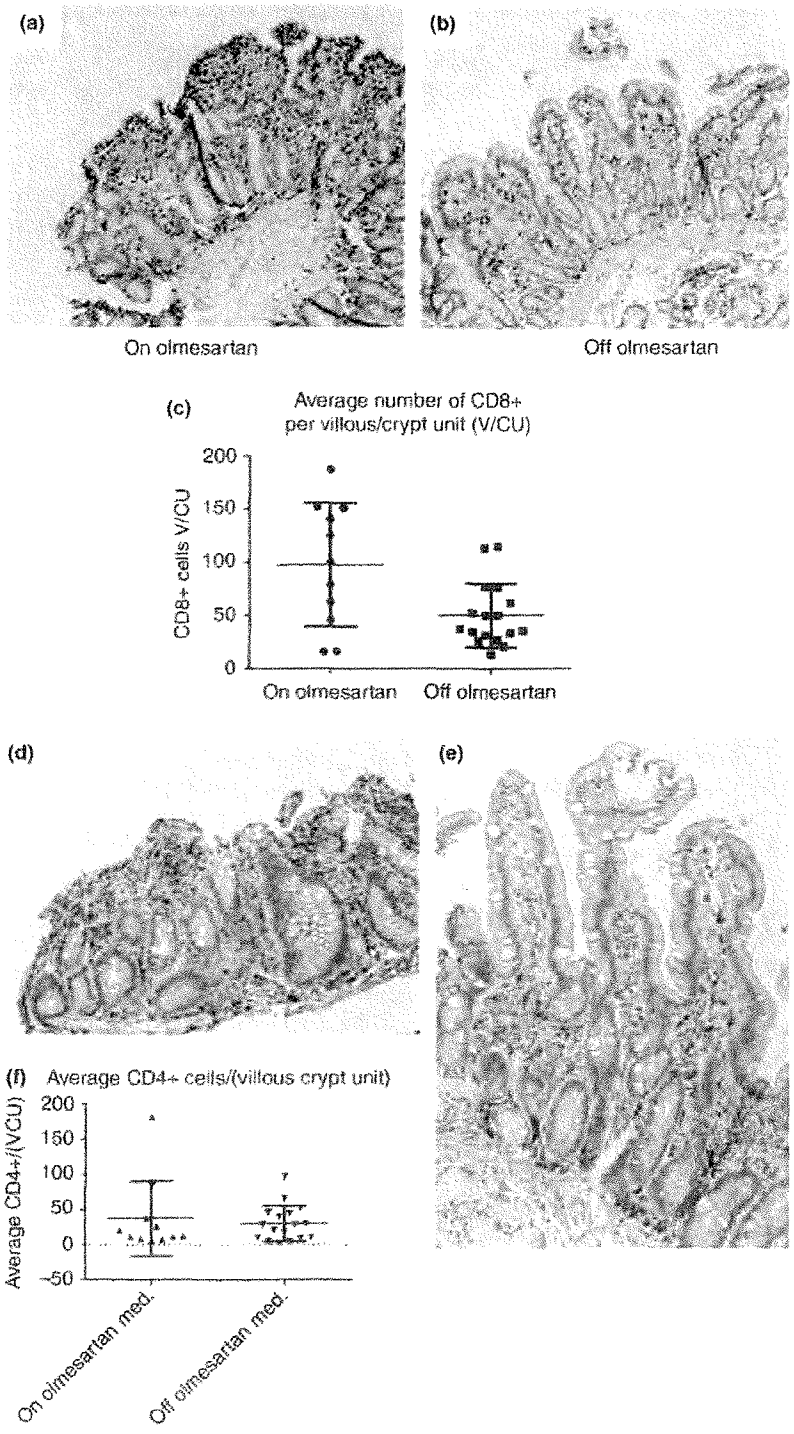

Figure 1 | *Distribution of* CD8+ and CD4+ cells in duodenal biopsies: Biopsies were extracted from the duodenum of a representative OAE patient while on olmesartan medoxomil (a) and off olmesartan medoxomil (b). Anti-CD8 staining (brown *colour*) is depicted *in* (a) *and* (b) (10× magnification). The average total number of CD8+ cells in each villous/crypt unit was counted for unpaired samples and graphed in (c), which displays the mean and s.d. of the CD8+ cells per villous/(crypt unit) for unpaired samples from patients on or off olmesartan medoxomil. A significant increase in the number of CD8+ cells occurred while on olmesartan medoxomil ($P < 0.05$ unpaired *t*-test with Welch's correction). In addition, biopsies were also stained with anti-CD4 (brown colour, panels d and e) (20× magnification). (f) The mean and s.d. of the number of CD4+ cells in each villous/crypt unit for unpaired samples from patients on or off olmesartan medoxomil. There was no significant change between on or off olmesartan ($P = 0.67$) using the unpaired *t*-test with Welch's correction.

unpaired *t*-test with Welch's correction), despite the concurrent inflammation while on olmesartan medoxomil. This increase in FoxP3+ cells with olmesartan medoxomiluse would indicate that the FoxP3+ cells were not able to effectively suppress the inflammation induced by olmesartan medoxomil.

TGFβ signalling through phosphorylation of smad 2/3

One method by which regulatory T cells are rendered nonfunctional, is the disruption of the TGFβR signalling pathway. Phosphorylated smad 2/3 (psmad 2/3) localised within the cell indicates that the TGFβ signalling pathway is activated, and nuclear translocation of psmad 2/3

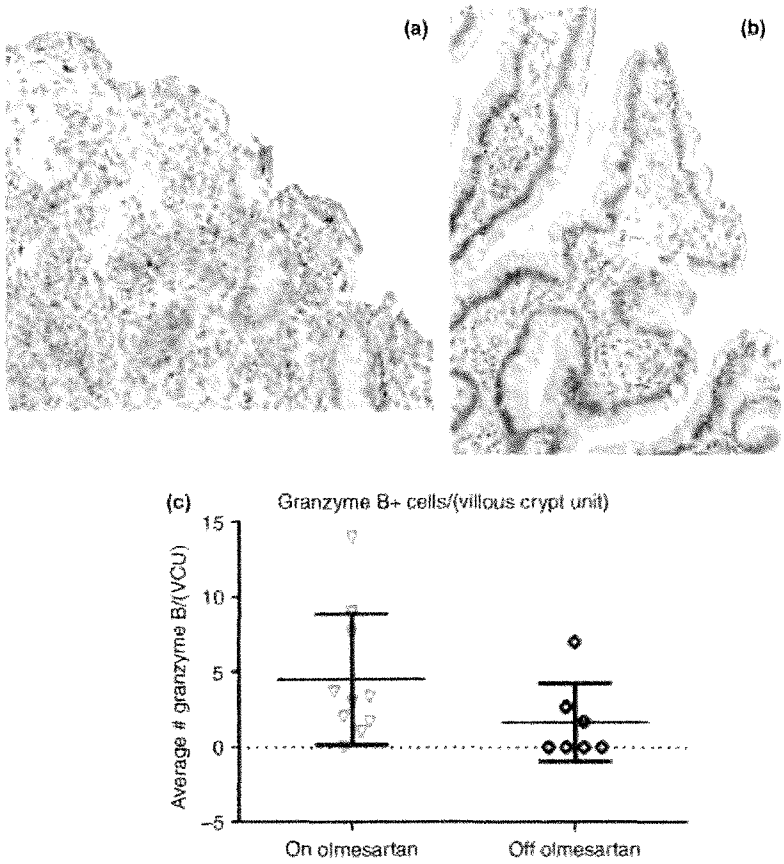

Figure 2 | Granzyme B expression in duodenal biopsies: Duodenal biopsies from a representative OAE patient while on (a) and off (b) olmesartan medoxomil were stained with anti-granzyme B (brown). (a, b) Taken at 20× magnification. The average total number of granzyme B+ cells in each villous/crypt unit was counted for unpaired samples and graphed in (c). (c) The mean and standard deviation. There was a numerical increase in granzyme B+ cells, but this was not statistically significant ($P = 0.1$) using unpaired *t*-test using Welch's correction.

indicates successful signalling. Staining of the duodenal biopsies for psmad 2/3 found that nuclear localisation was at the same level in both of the groups (Figure 4a, b). Intracellular localisation on the other hand was significantly increased while on olmesartan medoxomil as compared to off olmesartan medoxomil (Figure 4c,d) ($P < 0.01$). This would indicate that the TGFβ signalling pathway is active in the patients while they are taking olmesartan medoxomil.

Expression of IL-15R

In refractory coeliac disease, CD8+ T cells are rendered less sensitive to regulatory T-cell suppression, due to an overexpression of IL-15.[17] In addition, coeliac patients have an overexpression of IL-15 and IL-15R, which contribute to the activation of CTLs and subsequent killing of epithelial cells.[18] To determine if IL-15R expression was altered with the use of olmesartan medoxomil, immunohistochemistry staining for IL-15R was performed on duodenal biopsies of OAE patients. As Figure 5a,b demonstrate, IL15R is up-regulated while on olmesartan medoxomil, as compared to off olmesartan medoxomil, which is similar to coeliac disease. Figure 5c shows that IL15R is not significantly increased by lamina propria cells ($P = 0.2$), but is significantly increased by epithelial cells (Figure 5d) ($P < 0.05$), while on olmesartan medoxomil.

Response of Caco2 cells to olmesartan medoxomil

Because many OAE patients were diagnosed with colitis, we used a colonic epithelial cell line (Caco 2) to determine if olmesartan medoxomil could directly impact the function of colonic epithelial cells.[1, 3] To do this, Caco-2 cells were treated with olmesartan medoxomil, losartan or telmisartan. As one feature of coeliac disease is aberrantly increased production of IL-15, we first determined if exposure of Caco 2 cells to these ARBs results in the expression and/or release of IL-15. Olmesartan medoxomil at 30 mmol/L clearly induces the expression and/or release of IL-15 after a 4 h exposure (Figure 6a), whereas losartan (Figure 6b) and telmisartan (Figure 6c) at 30 mmol/L do not. We also did not see any expression of IL15 with treatment of the diluent for olmesartan alone (data not shown). Caco-2 cells also increased their expression of IL15R after exposure to olmesartan medoxomil (Figure 6d), but not after exposure to losartan

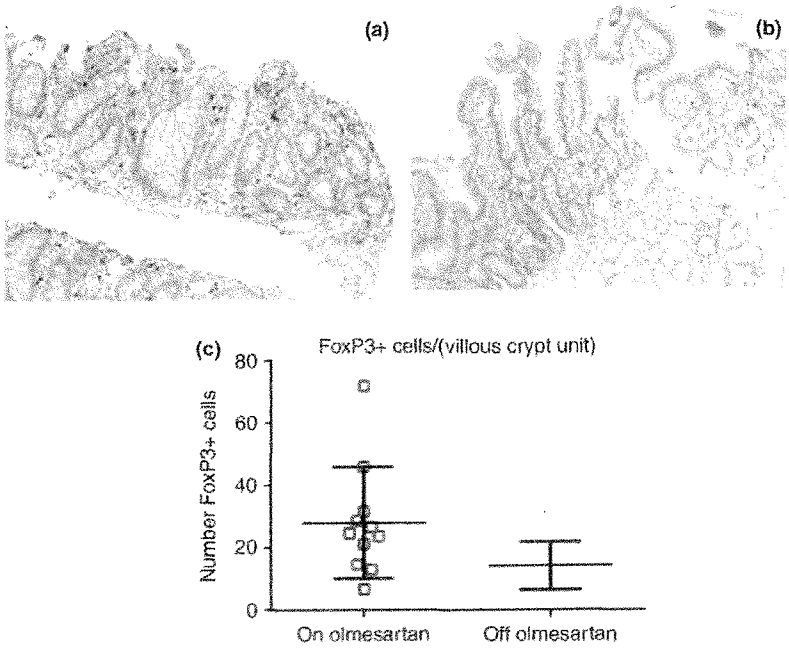

Figure 3 | FoxP3 expression in duodenum: Duodenal biopsies from a representative OAE patient while on (a) or off (b) olmesartan were stained with anti-FoxP3 (brown). (c) The mean with s.d. of unpaired on and off samples. There was a statistically significant increase in the number of FoxP3+ cells with the use of olmesartan medoxomil ($P < 0.05$), using the unpaired *t*-test with Welch's correction.

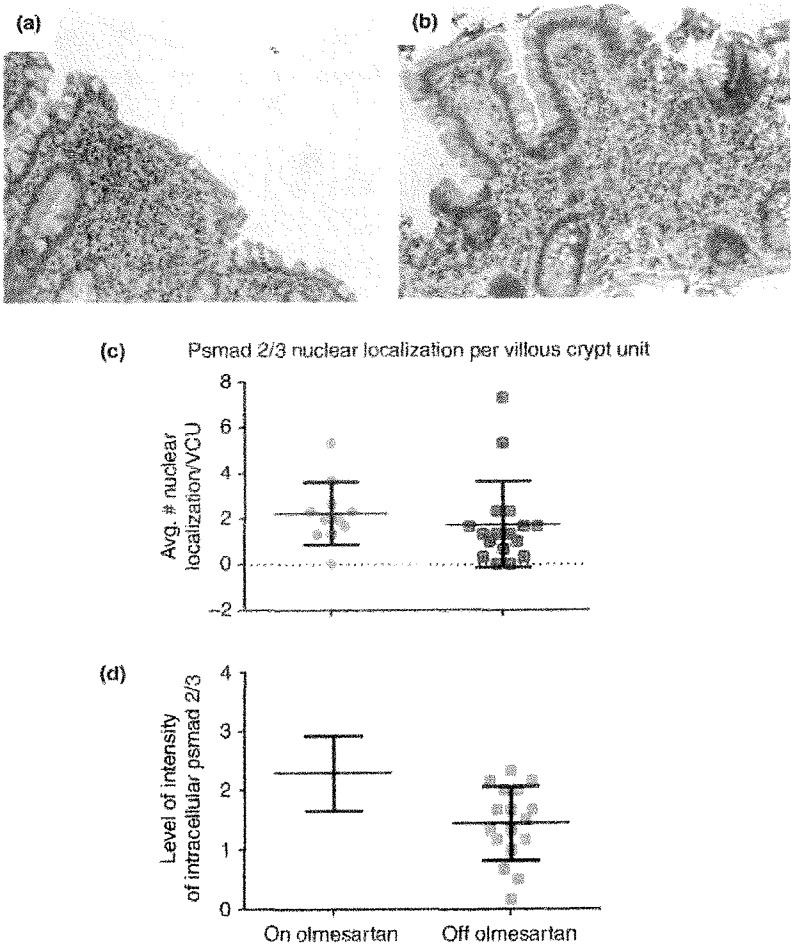

Figure 4 | Phosphorylation of smad 2/3: Duodenal biopsies from a representative OAE patient while on (a) or off (b) olmesartan medoxomil were stained with anti-psmad 2/3 (brown). (c, d) The mean with s.d. for nuclear (c) and intracellular (d) localisation of unpaired duodenal biopsies from patients on or off olmesartan. There was no statistically significant difference with the nuclear localisation between on and off olmesartan medoxomil ($P = 0.43$); however, there was a statistically significant increase in the intracellular localisation with the use of the drug, using unpaired *t*-test with Welch's correction ($P < 0.01$).

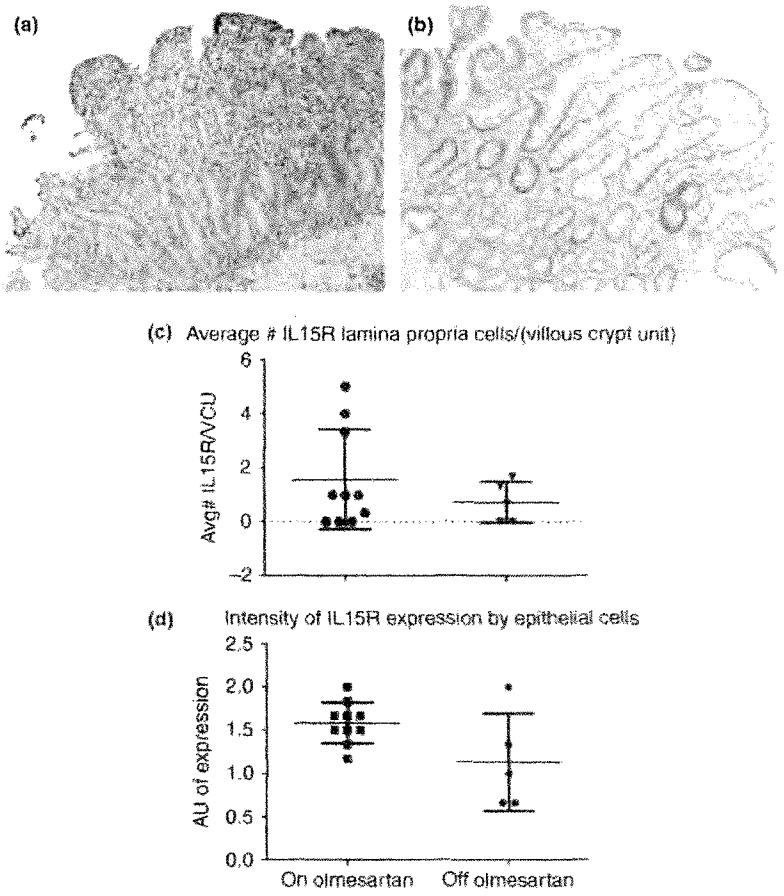

Figure 5 | IL15R expression/distribution: Duodenal biopsies from a representative OAE patient while on (a) or off (b) olmesartan were stained with anti-IL15R (brown). (c) The average number of IL15R+ cells in the lamina propria of unpaired duodenal biopsies from patients on or off olmesartan, while (d) shows the average intensity of anti-IL15R staining of the epithelial cells of unpaired duodenal biopsies. The observed increase in the lamina propria with the use of the drug was not statistically significant ($P = 0.2$) using unpaired *t*-test; however, the increased intensity of staining of the intestinal epithelium was statistically significant ($P < 0.05$) using unpaired *t*-test.

(Figure 6e). Untreated Caco-2 cells had the expected distribution pattern of ZO-1 (Figure 7a), which was somewhat altered at 30 min after treatment with olmesartan medoxomil (Figure 7b), and clearly disrupted after 4 h (Figure 7c).

Response of Caco 2 cells to olmesartan acid and diacetyl (medoxomil)

As displayed in Figure 8, olmesartan acid alone was also able to induce increased expression of IL-15 by Caco-2 cells (panels a and b). Treatment with the diluent alone, DMSO, did not induce expression of IL-15 (panel a), but olmesartan acid did (panel b). Corresponding staining of IL-15 after treatment with olmesartan medoxomil is provided as a direct comparison (panel c). As compared to DMSO alone (panel d), olmesartan acid was also able to disrupt the ZO-1 tight junction protein pattern (panel e). Staining with diacetyl (medoxomil) did not result in a disruption of ZO-1 (panel f). Using ImageJ software, the immunofluorescence was quantitated, and this is displayed in panel (g).

DISCUSSION

Patients with OAE have often been misdiagnosed with coeliac disease or more specifically refractory coeliac disease, due to the failure to respond to a gluten free diet. Our data show that OAE shares many of the pathogenic pathways present in coeliac disease. In OAE, there is a clear increase in CD8+ cells while the patient is taking olmesartan medoxomil. The additional increase in the number of granzyme B+ cells would indicate that CTLs are increased in the villous crypt units of the patients while on olmesartan medoxomil and may play a role in the destruction of the epithelium, especially as granzyme B+ cells are increased in both the lamina propria and the epithelial layer while on olmesartan medoxomil. This increase in granzyme B+ cells is similar to untreated coeliac patients and refractory coeliac patients.[19]

In addition, no significant change in the number of CD4+ cells was observed between those on olmesartan medoxomil and those off. In coeliac disease, gluten specific CD4+ T cells develop and expand, leading to

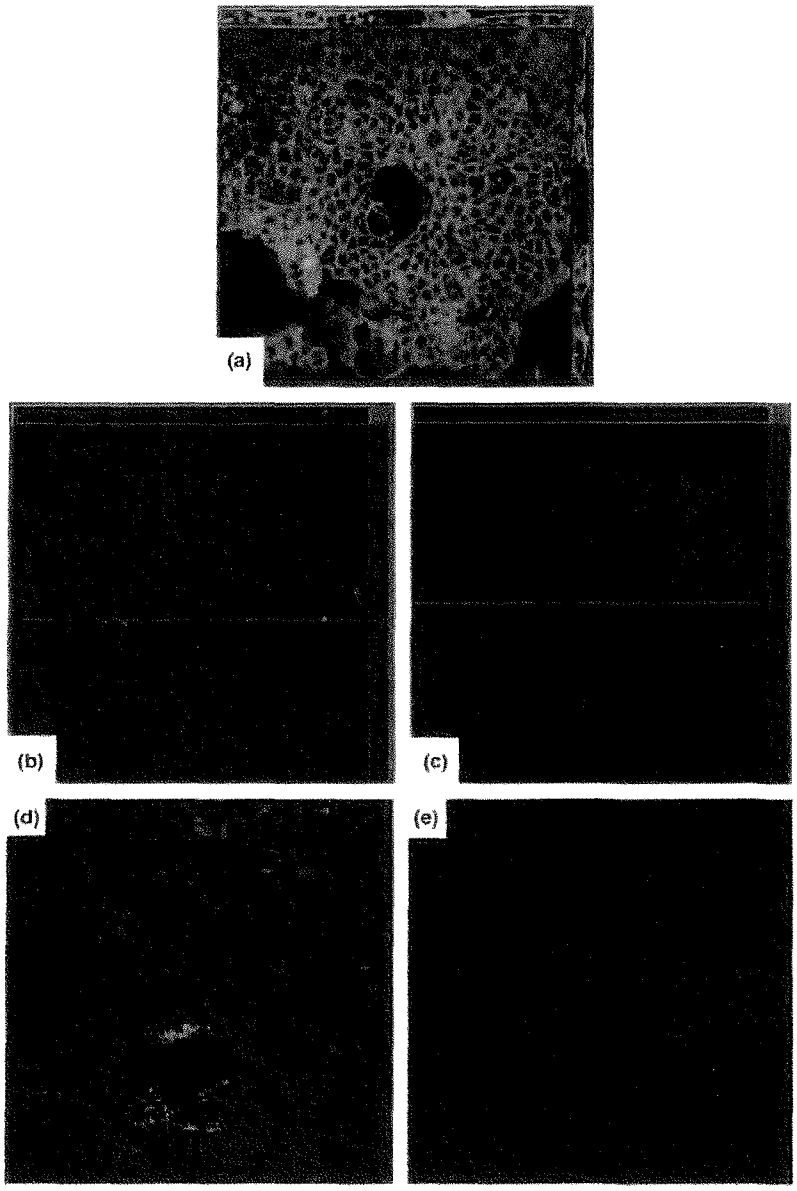

Figure 6 | IL-15 and IL15R expression by olmesartan medoxomil-treated Caco-2 cells. Caco-2 cells were cultured for 5–7 days and then treated with 30 micromolar olmesartan (a), 30 μmol/L Losartan (b), or 30 μmol/L Telmisartan for 4 h (c). Confocal laser microscope orthogonal images of anti-IL-15 staining are depicted, with anti-IL15 (green) and DAPI (blue). Magnification 40×. In addition, Caco-2 cells were cultured for 5–7 days, treated for an additional 60 h with 30 μmol/L olmesartan medoxomil (d) or losartan (e) and then stained for IL-15R (green).

increased levels of interferon gamma (IFNγ) in the intestine and anti-gluten antibodies in the serum.[20] The activation and expansion of inflammatory CD4+ T cells is mediated by HLA DQ2 and/or HLA DQ8, and as such, over 95% of all coeliac patients are either DQ2 and/or DQ8 positive. At some point afterwards, B cells begin to produce anti-tissue transglutaminase IgA (IgG in IgA deficient individuals). So far, no OAE patients have been identified that are positive for antibodies against tTG.[1, 13] Also of interest, was the high incidence of DQ2+ individuals in OAE (71%). Thus, although the number of CD4+ cells in the villous crypt unit did not change on or off olmesartan medoxomil, it is still possible that DQ2 could play a role in the pathogenesis of OAE. However, we continue to identify OAE patients that are neither DQ2 nor DQ8 positive (>4). Therefore, DQ2 positivity is not required for developing OAE.[1]

The ongoing inflammation suggests a loss of regulation of inflammation; the observation that FoxP3+ cells were significantly increased in the lamina propria of individuals on olmesartan medoxomil suggests that regulatory T cells were present, and indeed expanded, but lacked the ability to suppress the inflammation. Immune regulation in the intestine is crucially dependent upon TGFβ, and it has been speculated the ARBs may inhibit TGFβ signalling. Our results with the psmad 2/3

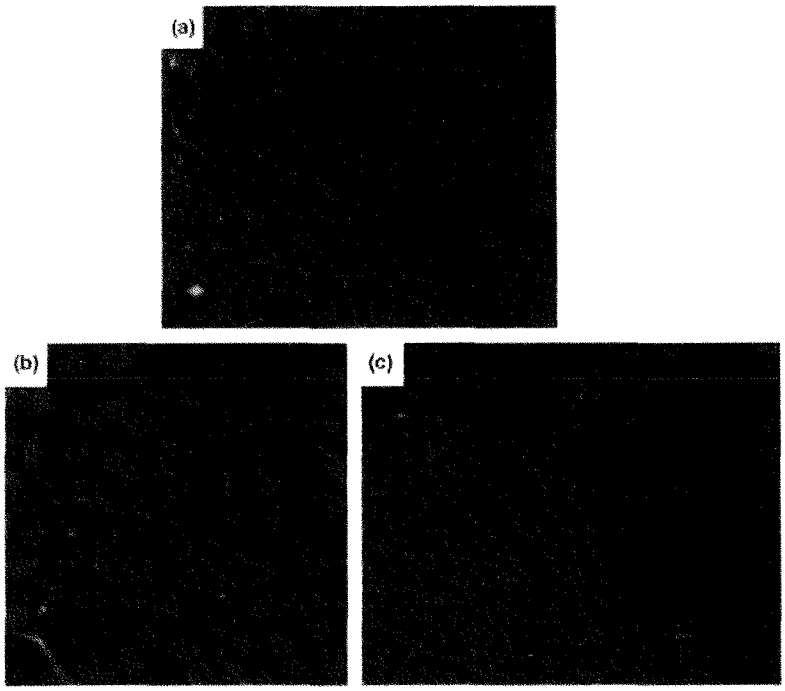

Figure 7 | Redistribution of tight junction protein ZO-1. Caco-2 cells were cultured for 5–7 days and stained for ZO-1 (a). (b, c) Caco-2 cells treated with 30 μmol/L olmesartan medoxomil for 30 min (b) or 4 h (c). FITC conjugated anti-ZO-1 and DAPI (blue) were used for staining. Magnification (40×). ZO-1 is depicted by green, and nucleic acid by blue.

staining, in which there was no difference in psmad 2/3 nuclear localisation between the on and off groups would suggest that TGFβR signalling is occurring at the same level on or off olmesartan medoxomil, implying that TGFβR signalling is occurring correctly and that olmesartan medoxomil is not inhibiting TGFβ signalling.

An additional pathway in which lamina propria lymphocytes are rendered unresponsive to regulatory T cells is the IL-15 pathway. Previous studies have demonstrated that overexpression of IL-15 and IL15R occurs in refractory sprue patients and overexpression of IL-15 in mice results in enteropathy that is diet independent.[18, 21, 22] The enteropathy that occurs in the transgenic mice that overexpresses IL-15 in the intestine is also associated with the influx of a large number of CD8+ cells.[22] Another publication demonstrated that in coeliac patients, IL-15 interferes with the suppressive ability of regulatory T cells.[23] Gluten can stimulate epithelial cells to over express IL15, and may be why coeliac patients as a group overexpress IL-15 and/or have sensitivity to IL-15.[23, 24] Disruption of the tight junction complexes of epithelial cells through disruption of ZO-1 localisation, also occurs as an innate immune response to gluten in coeliac disease.[25] Our observation that olmesartan medoxomil can increase the expression of both IL-15 and IL15R by Caco2 cells, and that OAE patients on olmesartan medoxomil have increased levels of IL15R would suggest that the enteropathy associated with olmesartan medoxomil use is a consequence of increased IL15 expression induced by olmesartan medoxomil. All together then, many of the mechanistic pathways present in OAE pathogenesis are similar to those of innate immune responses to gliadin in coeliac disease.[26] These pathways would include the increased numbers of CD8+ cells, the increased expression of IL-15R, and a state of nonresponsiveness to increased numbers of regulatory T cells. A central thread to all of these mechanisms is the intestinal epithelial cell, which is targeted by CTLs in coeliac disease.

In addition, our observations that directly treating Caco 2 cells with telmisartan and losartan neither increased IL-15 production nor disrupted tight junction protein complexes indicate that these deleterious OAE effects are not associated with all of the ARBs. This is supported by the findings of one study that found that telmisartan and losartan do not appear to be associated with enteropathy.[27] Further, the fact that olmesartan acid by itself can disrupt Zo1 in intestinal epithelial cells as well as induce increased expression of IL15, and that medoxomil by itself (diacetyl) does not, would suggest that olmesartan acid by itself is causing the pathology in OAE. However, many more experiments need to be done to prove that only olmesartan acid is causing all of pathology in OAE and to determine if any other ARBs exert any of the pathology associated mechanistic pathways induced by olmesartan acid.

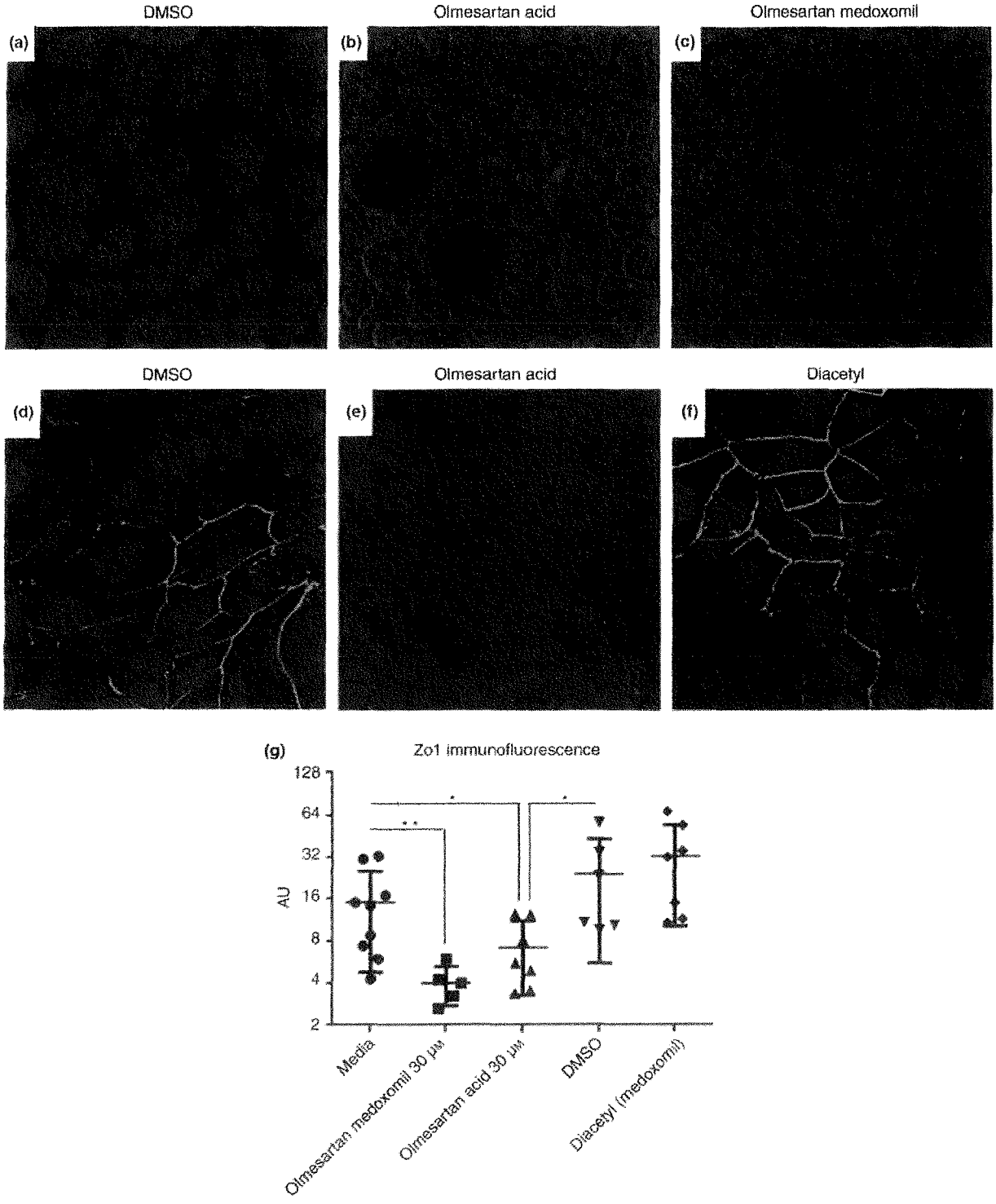

Figure 8 | Olmesartan acid alone can increase IL-15 and disrupt ZO-1. Caco-2 cells were cultured for 5–7 days, treated with 30 μmol/L olmesartan acid, 30 μmol/L DMSO, and/or 30 μmol/L diacetyl (medoxomil) for 4 h and then stained for IL-15 (red) (a–c) or ZO-1 (green) (d–f). (a, d) DMSO (diluent for olmesartan acid) alone. (b, e) Olmesartan acid and (c) olmesartan medoxomil. (f) Diacetyl alone. (g) A graph displaying the immunofluorescence of ZO-1 staining of Caco2 cells treated with the different reagents, where each dot represents a different well of Caco-2 cells treated with the listed reagent. $^{*}P < 0.05$ and $^{**}P < 0.01$.

In summary, a small number of patients will develop enteropathy in response to olmesartan medoxomil; this enteropathy is not gluten dependent, and both the stomach and colon of many OAE patients are also affected in addition to the small intestine. Our work suggests that epithelial cells respond to olmesartan medoxomil, and more specifically, the olmesartan acid portion of the olmesartan medoxomil increases the expression of IL-15 and disrupts the tight junction protein ZO-1. As some studies have demonstrated that refractory coeliac sprue patients also have aberrantly high expression of IL-15, one potential unifying theory for OAE is that these patients in certain circumstances were unable to down-regulate the IL-15 expression induced by olmesartan medoxomil, and therefore later developed enteropathy.

One limitation was our inability to safely rechallenge the patients with olmesartan medoxomil due to the severity of the illness; therefore, we cannot conclusively state that olmesartan medoxomil use directly causes the increase in the numbers of CD8+ cells that we had observed in OAE patients while on olmesartan medoxomil. We were also unable to do paired analyses because of the small number of cases in which paraffin embedded tissue was still available for research use from both on and off olmesartan medoxomil. We continue to identify patients with OAE and are conducting further analyses on other potential genetic causes as well as the mechanistic pathways that contribute to CD8+ cell recruitment by olmesartan medoxomil-treated epithelial cells.

AUTHORSHIP

Guarantor of the article: Joseph A. Murray, MD.

Author contributions: EV Marietta, A Rubio-Tapia: Study concept and design; EV Marietta, A Nadeau, AK Cartee, I Singh, A Rubio-Tapia: data acquisition; EV Marietta, A Nadeau, I Singh, RS Choung, I Singh, T-T Wu, A Rubio-Tapia, JA Murray: data analysis; EV Marietta: manuscript drafting; A Rubio-Tapia, JA Murray: critical revision of manuscript; EV Marietta, RS Choung: statistical analysis. All authors reviewed and approved the final manuscript.

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